An meinen lieben Vater

mit herzlichstem Weihnachts-Gruß

1907

Paul

TEMPORARY ALTERATION OF CHARACTER OF AN ORGANISM BELONGING TO THE COLON GROUP.

OSKAR KLOTZ,
Late Governor's Fellow in Pathology, McGill University, Montreal.

IN the spring of 1904 I was given the opportunity, through the kindness of my chief, Professor Adami, to study an organism isolated by him from the water of the St. Lawrence River. The results of these studies were published in the *Journal of Medical Research*,[1] and several interesting points in regard to the interagglutination of the Coli-Typhoid group were noted.

However, aside from the agglutination phenomenon, a peculiarity in the cultural characteristics was also observed. The microorganism on solid media resembled very much the appearance of colonies of B. coli. Grown on broth the microbe gave a stringy deposit, difficult to break up on shaking, and becoming more stringy on longer incubation. In litmus milk there was a primary acidity with a subsequent alkaline reaction of the medium, but no coagulation of the milk occurred. Indol was produced only after some weeks' incubation in Dunham's broth; and of the sugar broths, gas was produced most abundantly in the glucose medium. As was noted in my publication, the organism did not ferment lactose or saccharose when first isolated from the water, but did so after it had remained on the medium for some time. The saccharose broth was found to be more easily decomposed than the lactose, the latter medium showing only a very little gas formation after several days' incubation.

After the organism had been cultivated on artificial media for some months, the following experiment was reported:

A lethal dose of the bacillus was inoculated into the peritoneal cavity of a rabbit, and after its death (which resulted in three days), cultures were again obtained from it. The appearance of the organisms and the cultural characteristics were those of the bacilli inocu , except that in the fermentation tubes there was a slight development of gas in the glucose broth, none in the lactose or saccharose. Transfers were made from these tubes into the respective sugar broths, that is, the glucose colony was transferred to glucose broth, the lactose colony to lactose broth, and the saccharose colony to the saccharose broth. After 24 hours' incubation there was an increased amount

[1] *Jour. Med. Res.*, 1904, 6, p. 475.

of fermentation in the glucose transfer, but still none in the lactose or saccharose. A second transfer was made, similar to the above, and now at the end of another 24 hours the glucose and saccharose broths were both fermented; no gas appeared, however, in the lactose. In both the glucose and saccharose there was also acid production. In the lactose broth no change was evident, though there was growth in the closed arm of the tube. Four days' incubation and transfer on lactose broth gave a small amount of gas formation, and transfers from this again into lactose led to its fermentation in 24 hours. The stock culture as control produced gas in all these sugar broths in 24 hours.

This feature of the organism, its variability in the power to break up certain sugars, presented two very interesting problems. First, are we justified in making an indefinite number of varieties of B. coli, depending on cultural characteristics which may be modified artificially; and secondly, in isolating from water an organism which in the first transfers does not ferment one or more definite sugars, but which, after remaining on artificial media for some time, acquires the property, can we conclude that the microorganism has recently had an animal host?

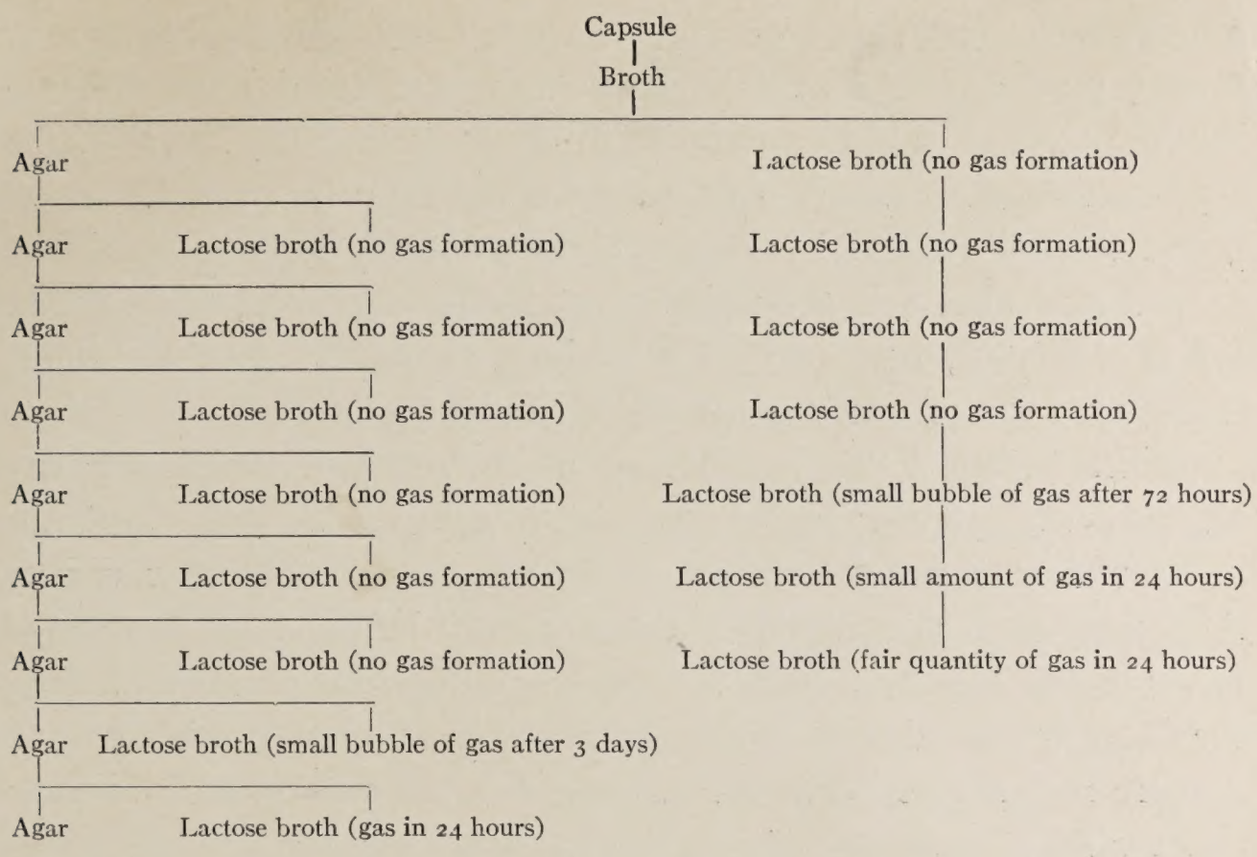
We have repeated our experiment of passing our microorganism, the Bacillus perturbans, through an animal. In this instance we made use of the celloidin capsules as devised by McCrae,[1] which we filled with a broth culture of the bacillus.

In the table it will be noted that two sets of transfers were carried forward, the one in which the parent culture was kept on sugar-free agar, and the other in which the parent stock was on a sugar medium similar to the transfer.

The sealed capsule was inserted, aseptically, into the abdominal cavity of a rabbit on September 19, 1904. The capsule was allowed to remain in the rabbit till February 10, 1905, in all 144 days. The capsule was then again obtained, and dropped for a moment without breaking into 10 per cent carbolic acid, after which it was placed in a flask of broth (without breaking), and incubated to insure against the chance of contamination. As no growth resulted, the capsule was ruptured and the microorganism was allowed to grow in weak broth for 18 hours. Transfers were then made into the various media, including sugar broths. The organisms resembled the original bacilli of the capsule in all the media except the sugars, though the growth was not in any case so luxuriant as transfers from the stock culture.

Transfers were made from and into the respective sugar broths daily. The glucose transfer showed a very slight gas formation on the first day, and this increased from day to day for several days. The first appearance of fermentation appeared in the saccharose on the fourth day, when it was only slight, and slowly increased with succeeding transfers. However, the lactose medium offered the greatest difficulty of fermentation, as can be readily appreciated from the following tab

[1] *Jour. Exp. Med.*, 1901, 5, p. 635.



As is seen in the above table, when the organism was transferred from a lactose to a lactose medium it regained its power to ferment lactose more rapidly than did the agar colonies. Having once acquired this property, the bacillus retained the lactose-splitting power in the successive transfers. In other words, the microorganism, having been deprived of one of its functions of altering the composition of certain materials by forced growth or environment, may again regain this function if it remain in contact with the material over an extended period.

Peckham[1] has given us the most complete study of the influence which environment exerts on the characters of organisms, especially of the colon group. In some cases this alteration consisted in an excessive activity of one function, in others the opposite, certain traits of the bacillus being entirely lost. In a series of cultural experiments, she was able to force B. typhosus to produce indol.

Of the external influences which can be brought to bear on bacteria, alteration of the quantity or quality of the food supply plays the most important rôle, and leads to modification of their biological nature. Thus some bacteria, in their normal metabolism, if we may so call the cell activity, secrete enzymes which split up proteids;

[1] *Jour. Exp. Med.*, 1897, 2, p. 540.

others secrete ferments acting on sugars. The colon bacillus, among others, possesses a proteolytic ferment, whose activity we estimate by the quantity of indol produced in the medium. If, however, the colon bacillus be grown over an extended time in river water, its power to produce indol is diminished or entirely lost; while again, as was said above, if a non-indol producing organism, such as the typhoid bacillus, be grown in a medium containing proteids alone, it acquires the property of producing indol.

Other examples of the influence of environment on bacteria are well known. Jenner[1] found that he could revert B. coli capsulatus to an unencapsulated form by cultural methods. The new variety then possessed characteristics dissimilar to the previous capsulated form; as for instance, while the capsulated bacillus coagulated milk, the unencapsulated stock lost this power when placed in this medium. A more remarkable difference was noted in the pathogenesis of these two varieties, for, as we know, B. coli capsulatus is very pathogenic for white mice, but becomes less fatal or even non-pathogenic on losing its capsule.

Experimenting with this same organism, B. coli capsulatus, Larulle[2] reports similar results of transforming his "opaque" variety into the "transparent," by passing the former through animals.

Other examples of alteration to a lesser degree in the characters of bacteria are seen in the everyday cultures, in the increase or decrease of the amount of acid produced, the morphological change which organisms undergo when inoculated on different media, and many other variations.

In a paper on the variability of bacteria Adami[3] discussed the alterations of character in bacteria due to environment, which give rise to different races of microorganisms. There he pointed out that two kinds of variations may occur, the temporary variation, in which the microorganism acquires characters that are lost after several transfers have been made, and the permanent variation, in which a new function or change is impressed on a microbe and remains with it in all future cultures. Of the latter class there are not many, for we must remember that what we call permanent is but a relative

[1] *Jour. Path. and Bact.*, 1898, p. 257.

[2] *La Cellule*, 1889, 5, p. 61.

[3] *Medical Chronicle*, 1892, 16, p. 366.

term. We speak of the characters as permanent when, after weeks, months, and years, no change is noted in the transfers from the type of the parent stock.

That at least temporary modifications can be brought about by such simple methods of cultivation and in so short a space of time seems to me to indicate that among those which we call varieties of B. coli there are some which owe their differentiating qualities to a prolonged habitat in a medium differing from that in which the parent stock has had its growth, and that through subsequent growth in suitable media the original qualities of the parent stock may be acquired. Our culture medium is at best a poor imitation of the natural habitat of these minute, and, I might say, impressionable, living bodies; hence we can conceive that investigators may obtain different results with the same organism. Thus with the colon bacillus it would seem that so long as we bring forward new sugars to ferment, we get an equal number of new varieties.

Further, when organisms, which under ordinary conditions produce gas in sugar media, are found to have lost this quality, it is one of the alternatives that the organism has been a parasite in the animal body. In our own case the same organism was also isolated from sewage flowing into the river, and the reactions of this strain of the microbe on media were the same as described, that is, it was primarily a non-lactose fermenter, but later acquired the property to break up this sugar.

STUDIES UPON CALCAREOUS DEGENERATION.[1]

I. THE PROCESS OF PATHOLOGICAL CALCIFICATION.

By OSKAR KLOTZ,

Demonstrator and Late Governors' Fellow in Pathology, McGill University, Montreal.

PLATES XXX AND XXXI.

INTRODUCTION.

It is a well known fact and a not uncommon occurrence that the interest in certain subjects or lines of thought fluctuates widely; we are ever too ready, when we think we have obtained an explanation of a certain phenomenon, to take much for granted and allow our enthusiasm to lapse. Such has been the case in the study of pathological calcification, which has gone through several periods of new theories and rest.

[1] This study was begun while the writer was Governors' Fellow in Pathology of McGill University, and has been continued and completed under a grant from the Rockefeller Institute for Medical Research.

Notwithstanding these theories, in reviewing the literature concerning calcareous degeneration or calcification, one is struck with the dearth of actual facts that are set forth upon which those theories are based. In truth the labor that has been spent in the actual study of the *process* has not been extensive and it is confined to a few papers in the last decade. There have been many who have reported the occurrence of calcareous degeneration of diverse tissues and have even gone minutely into the chemical nature of the organic salts present, but here the study has ended. Regarding the existence of pathological calcification we have abundant data, as to the process of calcification we know little.

Indeed, calcification is one of the commonest pathological conditions noted by the older writers, especially that occurring in the arteries and in fibromata of the uterus. Not infrequently the change in the arteries is spoken of as appearing "partly fatty and partly earthy." No doubt reference was made to the yellow atheromatous areas overlying calcareous plaques in the arterial wall. Not until Virchow in 1858 asserted that inorganic salts were deposited in supersaturated solutions of the same and that the deposit of calcium was of this nature, was there any discussion on the subject. Virchow held that calcareous deposits were chiefly found in old age and in diseases causing wasting of the bony framework, and that in this state calcium was dissolved out of the osseous system and on account of the too concentrated solution of calcium salts in the blood these were laid down elsewhere as metastatic deposits. In this way he explained the common occurrence in old people of calcareous plaques in the arteries.

Some years later von Recklinghausen reported the studies he had made upon the degenerations. He pointed out that calcium deposits result partly in tissues which are well supplied with tissue fluid, but also in tissues poorly supplied. When blood or tissue fluids are saturated with calcium salts, due to repeated breaking down of bony structures, he noted a secondary incrustation of the lung and kidney tissue. But there were other cases in which, as in fibroids and phleboliths, calcification proceeded under conditions of normal calcium content of the

blood. Von Recklinghausen looked on the processes of ossification and petrification as similar chemically, though differing biologically, in that the former took place in active and living cells, the latter in degenerating or dead tissues. He held that the factors governing calcification were: (i) the separation of sparingly soluble lime salts, (ii) the existence of these substances in excess in the fluids, and (iii) the presence of a foreign body which acts as a nucleus.

These factors would be of weight in considering fluids outside the body but not within. The lime salts in the tissue fluids are not found in excess during calcification and the common salts of lime found in the tissue fluids are very soluble. The deposit of calcareous plaques in the serous membranes and in the arterial walls he considered under ossification and as distinct from calcification.

Lancereaux's views were very similar to those of von Recklinghausen, though he attributed the supersaturation of lime salts in the blood to a hindrance of the excretion of these salts by the urine, and found them accumulated in the pyramids of the kidney and the mucosa of the stomach and intestines.

Thus it was that up to the time of Litten's ingenious experiments (1881), the theory of the excess of lime salts in the blood was accepted as explaining calcareous degeneration. No note was taken of the predilection of certain tissues for this deposit of lime, except that dead or dying tissues were regarded as a nucleus about which the carbonate and phosphate of calcium would crystallize. With Litten's studies—which will be discussed more fully later—a new theory was raised which superseded that inaugurated by Virchow, and up to the present time his has been the explanation of calcification accepted in the textbooks. After the appearance of Litten's work many followed in his footsteps, the majority supporting his views, although some have disagreed with him. The prevalent view, based on that of Litten, is briefly (dealing particularly with the cases of calcareous degeneration in the kidneys) that one or more irritants act on certain tubules in the kidney through which these irritants are excreted, and during their passage through the kidney cells,

so injure the tubular epithelium that a degeneration of the cell protoplasm occurs, and that this degenerated cell protoplasm has an affinity for, and fixes as an insoluble compound, the calcium salts brought to it by the lymph and blood. These, it is maintained, are the essentials for the deposit of calcium salts, and it is pointed out especially that a coagulation necrosis of the renal epithelium is a necessary stage in the process.

This view has been generally accepted; and yet it is noteworthy that no one has proved that outside the body there is to be obtained the formation, even temporarily, of compounds of lime and any form of proteid. The present investigation was undertaken, in the first place, to determine with what organic substance present in degenerating tissues it could be shown that lime salts entered into combination. It was in repeating Litten's and von Kossa's experiments and in studying also the changes occurring within celloidin capsules containing various substances, and introduced into the peritoneal cavity, that indications were gained leading to the results about to be detailed. It was first undertaken to produce, experimentally, calcification of the kidney and other tissues and to study the successive changes to be noted in these. The rabbit was most frequently used in these experiments, partly on account of the normally high calcium content of the blood, and partly because these animals stand various operations very well. But in addition many calcified tissues from the human body were studied, the process in connection with arterio-sclerosis especially, and, as will be detailed, not a few experiments of other nature were made during the course of the investigation.

In studying the calcareous degenerations, the tissues were subjected to a series of tests as to the staining qualities and the chemical nature of the material. From all the experimental work on animals and the analyses of human degenerating tissues, one conclusion was always arrived at, namely, that calcareous degeneration is preceded or accompanied by deposits of a soapy material. This soap exists in chronically inflamed tissues in advance of the deposit of lime, or, if the lime is already to be found in the tissues, the soap appears in the peripheral zone

of the calcareous infiltration. Moreover, it would seem that with the first deposit of soap, calcium salts are immediately attracted to it from the body fluids. Such progressive calcareous deposits are marked by the appearance in the periphery of groups of granules, some of which are found to react for both calcium and soap, others for soap only,—that is, some of the soap deposit has been converted into a compound containing calcium; in other words, calcium exists in the recently calcified areas as calcium soap.

That this calcium soap does not continue to exist as such was noted in old calcified fibroids and other tumors. In these it was seen that, except in the advancing border of calcification, the dense calcareous deposits are composed chiefly of calcium carbonate and calcium phosphate with no soap in its substance. It would seem that the fatty acid moiety of the calcium soap had here been replaced by the stronger phosphoric and carbonic acids to form the more stable compounds.

This is, in brief, the trend of the paper.

Before proceeding with the description of the work I wish to extend to Professor Adami my best thanks for the supervision of the entire work and for his unfailing help and the many suggestions which have been of great aid to me during the course of this and other researches.

STAINING METHODS.

Calcium and Calcium Compounds.—As a routine all specimens were stained with hematoxylin and eosin. These stained specimens gave the general character of the section, and, moreover, the presence of calcium salts, when in fair amounts, was indicated by the appearance of fine blue-black dust-like granules, which stained darker than other portions of the tissue and were easily recognized. The hematoxylin will, however, only stain calcified areas when calcium salts are present in very fair quantities; slight amounts are unrecognizable by this stain.

To demonstrate calcium salts in tissues we have two other methods, one of which was of the highest service in my hands.

If tissues containing lime salts be treated for five minutes with a solution of pyrogallic acid (1 to 40 in water), to which one per cent. of sodium hydrate has been added, the lime salts will be stained a dark seal-brown while the remaining tissue appears a light-yellow color. Except in tissues where large quantities of lime salts are present, the stain is unsatisfactory, just as in many cases which show the presence of calcium salts with the following method the pyrogallic acid treatment gave no results. The most satisfactory method we have used is that recommended by von Kossa, namely, the treatment of sections with a 5 per cent. aqueous solution of silver nitrate. This solution picks out the most minute quantities of calcium by depositing an intense black precipitate in its place. The sections were immersed in the silver solution for from 3 to 12 hours, then washed in distilled water, and mounted in the usual way in Canada balsam. As the silver treatment of sections consists of an impregnation and precipitate in the tissues, and not a stain, we obtain a much exaggerated picture of the actual quantity of lime salts present. However, this is of all the more service when minute quantities of earthy salts exist, and the method is not proposed as affording a quantitative measure.

As was pointed out by von Kossa, the blackening produced by the treatment of sections with silver nitrate is due to the production of silver phosphate which blackens in the presence of light; hence it would seem that a precipitation of silver is only obtained in the presence of a phosphate. The calcium deposit in the tissues exists, however, both as a carbonate and a phosphate, principally the latter, and this experiment would only lead us to detect the insoluble calcium phosphate. However, when sections containing calcium carbonate are treated for many hours with silver nitrate a certain portion of the two salts interact so that the calcium-carbonate granules become coated with a deposit of silver carbonate which in sunlight gives off carbon dioxide leaving the black silver oxide, or we can hasten the stage of blackening when some silver carbonate has formed by first thoroughly washing the specimen in distilled water and then treating it with a dilute soluble sulphide.

There is, however, I would point out, a means whereby the calcium existing in any favorable combination in the tissues can be demonstrated by the silver-nitrate method; and this consists in first converting calcium salts into the chromate by means of potassium chromate and then replacing the calcium by the silver, thus obtaining the silver chromate which blackens on exposure to sunlight. Under ordinary conditions the calcium chromate is soluble in water, but when tissues containing calcium are acted on in the cold we find that the calcium chromate does not leave them, and on further treating them with silver nitrate the red silver chromate is seen to become deposited in their sites. The use of silver nitrate in these impregnation tests is we have found facilitated by acidulating it with acetic acid. This same method demonstrates calcium when combined with fatty acids to form soaps.

I would point out that these micro-chemical tests for calcium do not demonstrate the actual calcium salts, but by replacement we demonstrate their presence and location with blackened silver salts.

Virchow and Halva have both analyzed calcareous nodules by chemically demonstrating the presence of both phosphate and carbonate of lime. However, they point out that the phosphate is much in excess; while Chiari found only phosphatic salts in the calcified nodules examined by him.

The method of staining lime salts with silver nitrate gave beautiful pictures in all of the tissues examined. Especially was this true of the sections of experimental calcification of the renal tubules (vide Fig. 2, Plate xxx.). It is true that the metals, mercury, copper, and lead, will give a black precipitate in tissues with silver nitrate, but they can be excluded in these cases when other evidences are given of the identity of the calcium.

However, in the experimental production of calcium deposits in the kidney we have to deal with another factor not often met with in the slowly forming calcareous nodules. In the former we are inducing a sudden change by means of chemical or mechanical reagents, and this has an effect on the general metabolism. Especially is this so in the inoculation of animals with such

poisons as corrosive sublimate, lead acetate, or copper sulphate. Here besides acting directly on the kidney tissue we produce an hæmolysis of the red blood cells and thus occasionally, depending on the strength of the inoculation, we find an iron deposit in the same convoluted tubules, which are the seat of calcareous degeneration. Similar though less amounts of iron can be demonstrated in the liver; but that the presence of iron in the kidney is not the cause of the silver deposit, is to be ascertained from the fact that no calcium reaction results in the liver. The iron in both instances is detected by the Prussian-blue test. Attention has already been called by Gierke to the occasional presence of iron in specimens of pathological calcification.

In the routine examination of aortas we were struck by the frequency of stainable calcium salts found in either the media or intima where no lesion was suspected from the macroscopic or ordinary microscopic examination.

Fats and Fatty Compounds.—For the detection of fats, fatty acids, and soaps, I found that Sudan III gave the most satisfactory results. I have also used Scharlach R., which is a very closely allied stain to Sudan III, but found a difficulty in the employment of it as the stain precipitates very readily in its crystalline form into the tissues. Scharlach R. is the more intense but cannot be used in saturated solutions. Osmic acid impregnation of tissues to demonstrate fats is of more limited use, as we know that only the oleic acid and olein are blackened by it, while stearic and palmitic acids with their respective fats are left untouched. Hence in this work, outside of making comparisons of the quantities of olein or oleic acid and the total quantities of fats and fatty acids, sections were stained with Sudan III; osmic acid was used only very occasionally. The nature of Sudan III and Scharlach R. is such that they are insoluble in water but are soluble in alcohol, chloroform, ether, fats, fatty acids, and soaps. A saturated solution of the stain is readily obtained in 70 per cent. alcohol, and as the fats, fatty acids, and soaps are more ready solvents of it than is 70 per cent. alcohol, fat in sections treated with a saturated solution of Sudan III will pick out the stain while the rest of the tissue remains

untouched. Our sections were usually stained 12–24 hours in the cold. (Vide Fig. 1, Plate xxx.)

Hence in Sudan III we have a stain which will differentiate fats, fatty acids, and soaps from other products in tissues. That soaps are ready solvents for Sudan III is readily demonstrated by making a concentrated watery solution of a soap and then adding the powdered Sudan III. The resultant mixture is a clear, deep red solution which can be filtered and further diluted. Such a stained though highly dilute solution, when evaporated on a slide gives a pinkish-yellow color, which is distinct from the golden-red staining of neutral fat. Fat particles even when emulsified to fine droplets retain this intense golden red appearance. The difference between the Sudan III fat stain and Sudan III soap stain is quite readily recognizable so that the two can be detected in tissues. It is more difficult to distinguish the neutral fats from the fatty acids in stained smears under the microscope, though olein appears distributed over the slide as stained oil globules which are easily picked out. These latter can be further differentiated by treatment with osmic acid.

It is to be noted that fatty acids as such are infrequent in the tissues, except in the case of dead tissues, such as gangrenous areas and pus, or as in the process of splitting up of fats in what are termed fat necroses. It is obvious that living cells do not pick up fatty acids unless, according to Fischler, it be leucocytes, in which case we can at times demonstrate fatty particles in the cytoplasm and show the vacuoles about the particles to be of acid reaction.

Fischler is most emphatic in his assertion that Sudan III is soluble in soap solutions, and the color produced on solution distinguishes this substance from the fat when stained in the same way, or, in other words, that fat and soap granules are to be differentiated in stained tissues. My results wholly confirm these statements. He states, further, that soap, when stained with Sudan III and then treated with 70 per cent. alcohol, immediately loses its color, the dye being taken up by the alcohol. This I failed to confirm wholly.

The statement is true of soap preparations in the test-tube, but, as we shall see later, soaps present in the tissues do not dissolve so readily in weak alcohols; hence they retain the stain after this treatment. As will be pointed out, these intracellular soaps do not exist as a free product, but form combinations with albumin with coincident alterations in reaction.

Calcium soaps are also stained by Sudan III, giving color reactions similar to those of potassium and sodium soaps. Calcium soaps, appearing in the tissues as a new deposit in which there is no excess of free calcium salts, are not distinguishable from other soaps. But when a portion of the calcium is freed and deposited either as carbonate or phosphate, we find, among the Sudan-III-stained soap granules, other, crystalline, bodies, highly refractile and not absorbing the stain. The latter granules, it is found, will precipitate with silver-nitrate solutions. In tissues therefore it is possible to treat the sections first with Sudan III, and later with silver nitrate, demonstrating the presence of soap granules and also showing amongst these free calcium salts not associated with fats.

Some might be sceptical regarding the ability to distinguish the finest fat particles from the granules of soap stained in sections. Such scepticism we can override by another method. If the material be cut by the freezing microtome, the sections quickly dehydrated in 70 per cent. alcohol, then washed with petroleum ether, the tissue may be rid of all the microscopic fat. If now soaps be present, the soap reaction with Sudan III will still persist. I do not wish to say that the ultimate fat is removed from the tissue, but all the stainable fat is taken out, and the petroleum ether has been without action on the soaps. Rosenfeld and Dormeyer have pointed out that tissues which do not show fat microscopically, when washed with ether or petroleum ether, still contain a considerable quantity of fat present within the cells; but the combined fats of this order give no reaction with Sudan III; they may be neglected in this regard. Here it may be noted that even after decalcified tissues have been prepared in the usual way for cutting in paraffin, by dehydrating in absolute alcohol and clearing with xylol, sections will still stain

faintly with Sudan III in the regions of active calcification. I cannot but believe that in such cases we have to deal with soaps rendered less soluble by their combination with albumins (vide p. 659).

CHEMICAL METHODS.

The Determination of Soaps.—In our first experiments the method of Hoppe-Seyler was employed. The tissue under examination was finely ground up and extracted with boiling absolute alcohol. From the filtrate, evaporated to dryness, fats were later removed by ether or petroleum ether, and the residue was demonstrated to contain sodium and potassium soaps, along with small quantities of calcium soap. The residue on treatment with mineral acids yielded stainable fatty acids, all dissolving in water; the sodium and potassium soaps were precipitated with calcium chloride. As calcium soaps are so slightly soluble in alcohol, only small quantities of them were thus obtained and we had to rely on the flame test to demonstrate the calcium present.

The use by this method of hot alcohol for the extraction leaves it possible, perhaps, that a small amount of neutral fat may be converted into soap by means of the alkalies present in the tissues. As I was, however, dealing with calcified tissues, such as fibromata of the uterus, from which all the surrounding superfluous tissue had been removed and which were then washed with distilled water, I can hardly believe that sufficient sodium or potassium remained in the tissue to give rise to the formation of soluble soaps.

To obviate this possibility of error, the method was given up and the fats were extracted without the use of heat. After the material had been finely chopped it was dried over sulphuric acid and then extracted with petroleum ether in many relays, the powdering of the tissue being undertaken between each treatment with fresh petroleum ether. Dormeyer is very emphatic in his assertions that this method of treating tissues with ether, even when carried on for a long time, does not remove the last trace of neutral fats contained within the cell membrane. He

prefers to digest with pepsin and 0.5 per cent. hydrochloric-acid solution, and thus break up all the cell bodies, whereby he claims to obtain 8.5 per cent. more of fat.

As the analyses here undertaken were not of a quantitative but only of a qualitative nature, these minute estimations are of no bearing. However, the gross fats being once removed, the powdered residue was treated with hot acid alcohol to dissolve out the soaps (which had been unaffected by the petroleum ether), and a filtrate obtained. From the filtrate the soaps were precipitated with acid calcium-phosphate solution, filtered off, and washed with ether. In tissues actively undergoing calcification, this residue was obtained in fair quantity—in fact, in greater quantity than in densely calcified tumors in which the amount of the calcium salt was altogether out of proportion to the fatty acid. The alcoholic extract of the residue, after treatment with ether and petroleum ether, yielded small quantities of calcium soap, which we must remember is only very slightly soluble in absolute alcohol.

THE PROCESS OF DEPOSIT OF CALCIUM SALTS IN BONE.

In Normal Bone.—In the above description of the methods employed I have had to refer to the results obtained, and, if only in a general way, I have indicated that, in tissues undergoing calcification, fats, soaps, and insoluble calcium salts are found associated in varying proportions, according to the stage of the process. We will now turn to a more detailed study of the process, and first consider whether under normal conditions—when calcium salts are laid down in bone—there is to be made out this same association; whether, in short, ossification and pathological calcification are processes of the same order.

The phalangeal bone of an infant six days old was examined by sectioning on the freezing microtome and staining with Sudan III. The head of the bone was cartilaginous with a centre of ossification within it. The sections were allowed to stain for eighteen hours, but at the end of this time did not take the Sudan III stain in any part. Both the cancellous and the compact bone were devoid of anything resembling fats, fatty acids, or

soaps. The femur of a fœtal guinea-pig was next sectioned and treated in the same way and with like results; that is, we found that in normal growing bone there appears to be no stage in which fatty substances play any part in the deposition of the lime salts, and we are led to believe that this laying down of calcium salts in the bone is a secretory process on the part of the osteoblasts. Again, in sections of decalcified skull bone we found the matrix entirely devoid of microscopical fatty substance. The bone corpuscles, or lacunæ, remained unstained with Sudan III; nevertheless there was some neutral fat lining the Haversian canals and surrounding the contained blood-vessels. Thus we conclude that *the process of calcification in normal growing bone is a distinct process in which the specific cells of the part play the chief rôle in laying down the calcium salts.* There was nothing in the stained tissue which would lead us to believe that there is any connection between fatty substances and the advancing bone-formation at the epiphyseal line.

Pathological Bone Formation.—What is true with reference to normal bone was found to obtain also in new bone developed under pathological conditions. For this purpose we examined a small bony nodule developing in the tissues to the inner side of the lower end of the humerus, secondary to trauma and due apparently, as pointed out by Dr. Archibald, to a local separation of portion of the periosteum. In the second place, we examined a portion of a large nodular, pure osteoma of the left frontal bone, obtained from a man twenty years old, which had grown into the cranial cavity and had produced great atrophy of the frontal lobe. [Royal Victoria Hospital—P. M. Reports, No. $^{15}/_{05}$.] In neither of these specimens did Sudan III give any trace of the presence of fat or soap in connection with the more recently growing portions.

THE PROCESS OF DEPOSIT OF CALCAREOUS SALTS UNDER PATHOLOGICAL CONDITIONS.

In the Arterial Wall.—We now pass on to tissues which, under pathological conditions, undergo *calcareous degeneration.* Of

these first and foremost are the arteries. All specimens examined by us, coming from persons over twenty-five years of age, showed more or less change in the aortic wall. The change varies from the faint white streaks which are seen through the intima, to the condition in which calcareous plaques are present, with and without ulceration.

The most remarkable point that strikes one in staining a series of aortas by the methods described, is the frequent presence of recognizable amounts of calcium salts in the media of adults. (Vide Figs. 3, 4 and 5.) In the routine examination of eleven specimens of the aorta, from persons varying in age from twenty-two to forty years, which on macroscopical examination showed no lesion other than fatty plaques in the intima, it was found that calcium salts were present in abundance at one time in the media, at others in the intima. It was not infrequently seen that the media was affected by the calcium increase while the intima remained unchanged or was affected by a slight extension only of the process from the media. We hope to call fuller attention to this change in the media in a subsequent article. So far it would seem to have escaped notice.

The white plaques in the intima have long been noted by pathologists and have passed under a host of names. Gross, as early as 1845, spoke of the condition as "atheromatous" and "steatomatous," where he says the microscope "brings into view a multitude of crystalline plates and fatty granules with albuminous and oily particles." Coplin has collected cases in which the change in the arterial wall was noted as early as the second year of life, but it is not our intention to deal here with the frequency of this affection. What is to be noted is that all vessels with arteriosclerosis have stainable calcium salts in either intima or media to a greater or less extent.

In studying normal aortas, taken from young adults, we found that neither intima nor media was affected by Sudan III, while only a few fat cells were seen in the adventitia. That is, in a normal and healthy state the intima and media of the aorta show no "microscopic" fat, and, further, when such aortas are stained with silver-nitrate solution there is no staining or deposit of silver

in the tissues. Obviously the small amount of calcium salts and fat normally present in the tissues is not detected by our staining methods.

Sections were made from the iliac artery of an adult aged thirty years, which was apparently healthy on gross inspection, with the exception that here and there white areas of the size of a pin-head and not raised above the surface were present in the wall. The specimen was one which had been picked from a number as a sample of an apparently normal large vessel of adult life. When stained with hæmatoxylin, it was seen that the elastic layer did not follow the lumen of the vessel at an equal distance around the artery, but at the sites of the macroscopic fatty areas the intima was thickened towards and bulged into the media, and here the elastica was distorted and at times broken. Away from these isolated changes in the intima, the artery was found to be perfectly healthy. When stained with Sudan III, it was found that these thickenings showed the presence of numerous fatty granules, many of which stained an intense reddish-yellow. This fatty material lay in aggregated clusters of granular particles of larger and smaller size, situated chiefly at the border-zone of the intima, next to the media. While these fatty particles were seen in the nodular portion of the intima, the rest of the arterial wall was free.

In the healthy portion of the arterial wall the elastic lamina did not stain with Sudan III, and followed the lumen of the vessel at an equal distance and in a single strand. As we approached the intimal lesion, the elastic layer split longitudinally into a number of strands, which now, instead of retaining a regular wavy contour, became of unequal undulations and spread apparently into the media. The individual strands of the elastic lamina were swollen and fragmented, showing in places "a snapping" of the fibres. In this area of change in the elastica, the fibres were also seen to take the Sudan III stain, which became more intense and more granular as we approached the thickening of the intima. Immediately below the fatty deposits in the thickened intima, the elastic fibres were found much split up and composed of short, curly, Sudan-III-staining, strands.

When we come to examine closely the particles stained by Sudan III, we find them of different characters. First, we note the larger droplets or granules which are not perfectly round and which are isolated, or else we see several of these fusing into larger masses. The color of these is unmistakably that of neutral fats. Besides these we find many more smaller particles which are distinctly semi-crystalline and of a much lighter color, approaching almost to pink. These pink-staining particles are found in greatest abundance along the border-zone between the intima and media, and often occupying the elastic lamina itself.

It was found that when consecutive sections were stained alternately with Sudan III and silver nitrate, the silver nitrate was precipitated in areas identical with those picked out by Sudan III, though the relative quantities were unequal, there being proportionately more fat present. That is, the calcium deposit picked out by the silver nitrate occupies the nodular portion of the intima and is not seen in the rest of either intima or media. Further, the portion of the thickened intima picked out is that occupied by the pink-staining granules. In the less affected parts the calcium appears as fine dust-like material staining an intense black. Judged by the outline, seen as a silhouette against the lighter background, the individual granules here are crystalline. In places the elastic lamina was picked out as a wavy line composed of aggregated granules of calcium.

In this specimen we have the early change in the aorta, a condition which we would not yet speak of as arteriosclerosis. The white pin-head patches on the intima are seen to represent areas with fatty substance of two kinds: one an intensely yellowish-red staining material, which is fat; and lighter particles, which from their reaction with Sudan III can only be soaps; and, further, the thickenings in the intima are shown to contain an increase in the calcium content, and this increase to occur in conjunction with the light, soapy staining material. In other words, the calcium increases side by side with the soap; and the arrangement described suggests strongly that we are dealing with a deposit of calcium soap.

In another instance there was a generalized thickening of the

arteries, though only at the bifurcation of the aorta was any macroscopical calcareous degeneration to be seen. The aorta showed numerous white plaques raised above the surface, and one of these from the abdominal aorta was sectioned. Stained with Sudan III both media and intima showed numerous aggregations of stained particles; the staining in the intima was confined chiefly to the atheromatous thickening in which considerable fat-staining masses occurred, while outside the nodule the granules were collected mainly round the elastic lamina. As in the previous case the granules could be divided into two classes: the larger masses which fused with other contiguous particles, readily recognized as fat deposits; and the smaller, lighter-staining granules which appeared semi-crystalline in shape. In the intima the larger fat masses were in excess of the finer granulations, while in the media the reverse condition existed; that is, the light-staining granules were much in excess of the fat, in fact only a little fat was to be seen on the boundary between the media and intima. In the rest of the media there were clusters of fine sand-like particles lying between the elastic layers and forming rows, more or less wedge-shaped, which occupied the position of the muscular fibres. In the media the stained granules were intermingled with numerous unstained refractile bodies, which disappeared from the specimens when treated with acid solutions. On treating the sections with silver nitrate it was found that the blackened deposit was present in the media in the regions where the Sudan III demonstrated the light-staining soapy particles. Similar to the Sudan III granules, the silver-nitrate deposit was aggregated into clusters lying parallel to and between the elastic fibres. The silver-stained particles in these clusters were much in excess of the refractile bodies seen in the Sudan III specimens; they tallied in number with those seen in unstained sections. The distribution of the silver-stained calcium salts was fairly general through the media, the greatest amount being towards the internal surface. (Vide Figs. 3 and 4.)

In the intima, however, there was less calcium to be seen, that present being confined to the atheromatous areas where the deposit seemed to be invading from the media. Beyond the

atheromatous plaques, there was no lime salt to be seen in the intima excepting a fine, dust-like infiltration along the boundary zone. The whole condition in the arterial wall we look on as consisting of two distinct processes: the change in the media of a chronic nature and of longer standing than the intimal change, the degenerative process with fatty change having been progressive; and the atheromatous condition in the intima of more recent date, owing to which time had not been given for such extensive deposits of calcium salt.

We now undertook to remove all the neutral fats and fatty acids from the sections of the aortas above described by treating them with ether and petroleum ether. They were first washed in 70 per cent. alcohol before subjecting them to the ether. After this treatment the only material remaining which could stain with the Sudan III would be a soap. On examining the sections stained with Sudan III, it was found that all the bright-red staining neutral material had been removed and all that remained to take the stain were small crystals of a light salmon-red color. These crystals lie in clusters of 50 or more, the long axis of the clusters being parallel to the direction of the fibres. The clusters are to be found mostly in the intima at the atheromatous area and in this tissue lie some little way inwards from the endothelium. In the deeper parts of the intima the clusters become more broken up, and after staining with silver nitrate are seen to be mingled with calcium deposits, which are absent in the more superficial parts of the intima. Hence the Sudan-III-staining particles which remained near the boundary zone between the media and intima after treatment with petroleum ether are combined with calcium salts, while those, apparently of more recent origin, lying near the endothelial surface contain no calcium salt. When we examine the aorta beyond the atheromatous patch, we lose any evidence of soap in the intima except in the deeper portions near the media, and only as the atheromatous area is approached do the layers between the fibrous strands become widened and the spaces become the site of deposit of soap granules. In the media, where, as we have described, the calcium salts are much more marked, similar clusters of granules

to those noted in the intima are found, with, however, the difference that in the media the clusters are compounds mainly of silver-nitrate salts—much calcium with a little soap,—while in the intima the proportion is exactly reversed.

To resume, it may be stated that by employing suitable methods it is found that calcareous deposits occur in the arterial walls, more particularly in the wall of the aorta, at an earlier age than is usually suspected.

These deposits vary as regards the region first affected. This variation may be in regard (i) to the internal elastic lamina, (ii) to the media, more particularly its muscle fibres, and (iii) to the intima in areas of nodose sclerosis.

Judging from the treatment of sections for the demonstration of fats and calcareous salts, the stages in this process are (i) degeneration of the affected region with deposit of fatty globules, (ii) formation of soaps, mainly with calcium, (iii) deposit of calcium salt devoid of a fatty moiety.

The results obtained in connection with calcified deposits in the arterial walls were confirmed and extended by an examination of other conditions of calcareous infiltration.

Calcareous Infiltration of Thickened Pleura.—The cases examined were evidently of some duration. There were thickened fibroid adhesions between the visceral and the parietal pleuræ, and in the centre of the fibroid layers dense calcified plaques occurred. The plaques were found to form rather irregular laminæ with somewhat irregular surfaces. By examining the material after decalcification, the section through what was a densely decalcified plaque was found to be stained a diffuse pale-yellow with Sudan III, no individual granules being present; but in the immediate neighborhood of the plaque was an aggregation of masses stained deeply with Sudan III. Therefore at the periphery where the process of calcification was still continuing there was definite evidence of the presence of fats.

In studying the decalcified sections it was noticed, as frequently observed by others, that the decalcified areas stained more deeply with hæmatoxylin than does normal tissue. Very

few nuclei were recognized. The matrix left behind after removing the calcium salts was of a hyaline nature. The existence of this matrix led Litten and others to the theory that the calcium salts are deposited as calcium albuminates. That the fat or soapy substances, still exist in connection with the matrix is indicated by the light-yellow color given with Sudan III; and remembering that, as we have noted, soap solutions and albumins form insoluble compounds with which calcium can combine, it would seem more probable that we are again dealing in this instance with the resultant albuminous material deposited after the calcium has separated from the combination to unite with phosphoric and carbonic acids, and possibly to some extent also after a part of the soap has been split off to enter into more soluble combinations.

Calcified Fibromata.—A similar condition is found in calcified fibroma, in which the most extensive infiltration is not in the centre of the fibroid mass but in the outermost layer, whence it is proceeding towards the centre. In each of the specimens examined, three zones of change were well marked. In the outermost area (which varied in thickness), when the sections are stained with both Sudan III and silver-nitrate solution there is seen a dense black mass, the small amount of soap present in this zone being wholly obliterated by the black-stained lime salts. At the margin of this zone and farther inwards clusters of granules are to be seen, some of which stain with Sudan III, others with silver nitrate. At times an individual cluster, black at one end, yellow at the other, can be made out. In this area, between the groups of granules can still be seen the wavy fibres of connective tissue. This constitutes the second zone. The third zone, farther inwards, exhibits granules staining only with Sudan III. Thus there is well-marked fatty change, the presence of calcium soap, and finally the presence of calcium salts showing no soapy constituent.

That calcifying fibroid tumors should show a deposit of lime salts at the periphery is a phenomenon of the same nature as has been observed in connection with lithopedia and with kidneys undergoing calcification after complete cutting off of their blood

supply. The fibroid tumor that undergoes calcification is one that is undergoing necrosis through defective blood supply, and the calcareous salts deposited are not those already present in the tissue itself but those precipitated from the lymph which diffuses into the dead tissue from without. It follows that the diffusible salts are taken up by the first soapy substances with which they come into contact in this process of diffusion, and only when those in the peripheral zone become calcified do the soapy and fatty matters situated more deeply have the opportunity to form calcium compounds.

Calcareous Infiltration of Ovarian and Broad Ligament Cysts.—Cysts of this nature, in which a thin shell of lime salts is laid down in the fibrous tissues of the walls, are occasionally encountered. The process is usually confined to a layer bordering on the cyst cavity beneath the lining membrane of the cyst in the surrounding fibrous tissue. In these we have found the usual evidence of fatty change, of soap formation in the immediate neighborhood of the areas, and of deposition of lime salts.

Calcareous Tubercular Nodules.—The nodules can be decalcified, cut by the freezing microtome, and the sections treated with Sudan III or Scharlach R. It has been usual of late years to speak of tubercular caseation as a process of protoplasmic degeneration in which there is very little associated fatty degeneration. It is interesting therefore to note that by employing the methods described by us it can be demonstrated that the caseous areas which have undergone calcification contain a very considerable amount of fatty matter, so much indeed that the yellow staining areas of the tubercular nodule stand out very prominently from the surrounding tissue. Treatment of such sections with ether removes some of the material stainable with Sudan III, but by no means all. As might be expected from what has already been said, differences are to be made out between old and more recent calcified tubercular areas. In dense, old, calcified glands and nodules there is less of the fatty staining material left, and it is only here and there, where the soap moiety has not been wholly removed, that the deposition of the lime salts gives any results with Sudan III, while in softer and less

dense nodules larger and smaller granules staining with Sudan III are abundant. It is noticeable that very little fat is to be made out in the enveloping fibrous tissue. The question naturally arises, Where does this fat come from? Examination of lung tissue in cases of active tuberculosis showed that the fat and its products are already present in tubercles showing the early stages of central necrosis which as yet scarce show caseation. In caseous tubercles this fatty matter is still more evident, and in these also calcareous salts in the form of fine granules are to be detected. It was only in those tubercles in which the fatty substances were aggregated into larger granules that lime salts could be demonstrated. In the earlier non-caseous areas there was no reaction of the calcium; and it is to be noted that there were more Sudan-III-staining granules in the centre of the caseous areas than at the periphery. This series of events indicates the following stages, viz: necrosis, fatty degeneration, formation of calcium soaps, and deposition of calcium salts having no soapy moiety.

Pancreatic Fat Necrosis.—It has been pointed out by various observers that in pancreatic lesions followed by fat necrosis the neutral fats within the fat cells undergo decomposition, and as a result fatty acid crystals are left behind. These fatty crystals after a time unite with the lime salts of the tissue fluids to form insoluble calcium soaps. We found that in the experimental production of fat necrosis the fatty acid crystals could be stained readily enough, but unless the animals experimented upon remained alive for more than a week the lime salts were not to be demonstrated microscopically in the lesions. In other words, the combination of the fatty acids with calcium to form calcium soaps is not an immediate but rather a slow process. A definite period of time seems to be necessary before the calcium soaps accumulate so as to be present in stainable quantities. We examined one specimen of hæmorrhagic pancreatitis in man and found that fatty acid crystals had been deposited in the peripheral zone of the necrotic area. In this zone calcium salts were seen in small amounts distributed as a fine dust. The specimen, however, was an accidental find in a recent autopsy,

two small foci of necrosis in the tail of the pancreas, each scarce one centimetre across, being present, and there was no clinical indication of the length of time that the condition had existed. The earlier observations upon the presence of lime soaps in connection with pancreatic fat necrosis will be referred to later.

The preceding section of this paper may be reviewed as follows:

(1) All calcareous infiltrations are preceded by fatty changes and in them substances are found consisting of neutral fats, fatty acids, and soaps, which stain with Sudan III.

(2) By employing appropriate methods, the microscopic fat and fatty acids can be removed, the soaps remaining behind, the last being detected by reason of their differential staining with Sudan III.

(3) The granules in certain regions which give the soap reaction with Sudan III give also the calcium reaction with silver nitrate.

(4) As the process of calcification advances, many of the masses deposited in the part no longer stain with Sudan III, but only react for calcium salts.

In areas undergoing calcification three zones are to be made out: (1) The oldest zone of complete calcification, exhibiting dense calcareous deposit lying in a hyaline matrix; in this zone little soap or fatty substance is to be demonstrated. (2) The intermediate zone, in which granules of lime salts and soap granules are in close apposition; some of these granules give both the calcium and the soap reaction. (3) The most recent zone, which gives the reaction for fat, fatty acids, and soaps and fails to reveal calcium salts in sufficient quantities to be recognized under the microscope.

ON THE PRESENCE OF SOAPS IN THE ORGANISM UNDER PHYSIOLOGICAL CONDITIONS.

Since the observations above detailed indicate that soaps play a most important part in the process of pathological calcareous change, it appears desirable to compare with them observations

made upon the part played by the soaps in the physiology of the animal economy.

In 1857 Marcet stated that during fat digestion the fats are converted into soaps in the intestinal canal and these soaps are absorbed by the intestinal mucosa. The absence of any amount of soaps in the blood comparable with the amount of fat absorbed led others to conclude that the soaps as such did not pass beyond the endothelial cells, and to suppose that a glycerin molecule is furnished by these cells, the reconstructed fat being passed into the lacteals. Heidenhain pointed out that some at least of the fat is taken up by the leucocytes on the surface of the intestine and is carried by them directly into the lacteals and blood-vessels. More recently, Ramond has denied the validity of Heidenhain's observations, and has concluded that the greatest part of the fat absorbed enters the portal system and not the lacteals, and enters in the form of soap and not of fat. From this statement we infer that Ramond regards the soap as becoming transformed into fat during its passage from the endothelial cells to the portal capillaries. He finds also that the liver tissue contains a ferment capable of splitting fats into their respective fatty acids. Beneke and later Hoppe-Seyler have pointed out that the blood normally contains soap in solution. Beneke makes an interesting suggestion as to the manner of recovery from pulmonary fat embolism. He points out that possibly a solution of the fat of the emboli is brought about through the action of the lipase present in the blood, which converts the fat into a soluble soap. He also states that fat inoculated subcutaneously disappears so rapidly that the only satisfactory explanation is one based on its conversion into soluble soap.

Still more recently Schultz, who studied fats as a whole in the blood, found that no less than 28 per cent. of the total fatty acid obtainable is present in combination as soap. During starvation, when the fat deposits are used up, he found an increase of the fat in the blood and concluded that the fat is transported from its normal deposits by way of the blood. The fat in the tissue cells he holds becomes transformed into soap and thus passes in solution into the blood.

As to the manner of conversion of the fats into the fatty acid, in which form combination occurs with alkalies to produce soaps, we have the observations of Hanriot. This observer found that blood serum, pancreatic juice, and liver juice contain a ferment capable of converting the neutral fats into their respective fatty acids. This ferment was not present in muscle tissue, testicle, or thyroid gland. The substance isolated by Hanriot seemed by its action to be a true ferment, and was present in all the animals examined. He made a distinction between pancreatic lipase and serum lipase, claiming that they are different substances chemically. Cohnstein and Michaels, who also found a lipolytic ferment in the blood, differ from the former observer in believing that it is present in the red blood cells rather than in the serum.

There can be little doubt that the leucocytes and endothelial cells play parts in the synthesis of fat. It is well known that leucocytes are fat-carriers and that they transport fat from the intestinal canal during fat digestion. The quantity of fat transported must be very small indeed, but Arnold has found that subcutaneous injection of soap solutions into the backs of frogs attracts leucocytes which become filled with fat, and he also noted that eosinophilic leucocytes are especially active in this fat synthesis. Fischler has shown by similar experiments that sub cutaneous injections of soap are followed by deposit of fat in the organs, and that when a soap solution is passed through the renal vessels the fat particles can be seen between the endothelial cells and in the kidney tissue. Hence it would seem that endothelial cells take part in the conversion of soap into fat. This view is also supported by the statements of Ramond, namely, that the fat from the intestine passes through the intestinal mucosa, as through a dialyzing membrane, in the form of soap and is reconstructed on entering the portal system.

Munk studied the toxicity of soap and pointed out that soap solutions injected into the portal vascular system are only one-sixth as toxic as when inoculated into the peripheral vessels. From this it would seem that the portal endothelia or the liver parenchyma are especially active in converting the soaps into neutral fats.

THE PRESENCE OF SOAPS IN THE ORGANISM UNDER PATHOLOGICAL CONDITIONS.

It is not a little remarkable that very few observations upon the part played by soaps in pathological conditions have appeared in the literature. We have encountered isolated notes only upon the subject, and do not know a single research, properly so-called, in which their rôle has been fully worked out. Many years ago Virchow called attention to the presence of calcium salts in degenerating lipomata, and very correctly concluded that the fats present in these tumors combine with the calcium salts to form soaps. Kyber in 1880 referred incidentally to the part which both alkalies and fatty acids must play in the process of calcification. Somewhat later Jaeckle and others enunciated the theory that the formation of soaps is one step in the process of calcification of fatty tumors. But this theory has to our knowledge never been tested experimentally and would seem to have become obsolete. In 1890 Langerhans pointed out that in fat necrosis following pancreatic lesions crystals of fatty acids appear, and in many cases unite with calcium of the blood to form an insoluble calcium stearate which occurs at the site of the lesion. It does not appear that Langerhans ever continued these observations or expanded his findings so to apply them to calcification in general. Quite recently Fischler has published a preliminary note upon certain methods for detecting the presence of fats and soaps in the tissues; and though he does not state it definitely, he nevertheless seems to imply that his observations point to an important part played by soaps in more than one process.[1]

As indicated in the preceding pages, our observations all point to the fact that the formation of soaps is a necessary step in the development of pathological calcification. We may at this

[1] The number of the *Centralblatt* containing Fischler's article reached America, I may add, after I had forwarded to Philadelphia the abstract of my preliminary communication upon this subject, which was read at the meeting of the Association of American Physiologists in December, 1904. A study of Fischler's paper will show that he has reached his conclusions by the employment of methods differing from those given here.

point present our further studies on the chemistry of this process.

The analysis of aortas which were in the process of calcification yielded calcium soaps in small quantities together with sodium and potassium soaps, though chemical extraction did not give as clear an idea of the process as was obtained by treating sections with petroleum ether and later with Sudan III. Sections so treated seemed to indicate the presence of a fair amount of soaps. By analysis the quantities obtained were very small. Indeed the Sudan III stain after treatment seemed to show clearly that substances which *in vitro* are soluble are relatively insoluble when present in the tissues. For instance, sodium and potassium soaps are, as we know from every-day experience, easily soluble substances, while, on the contrary, calcium soaps are relatively insoluble. We were prepared, therefore, to find that the tissues contain relatively small amounts of the former, and large amounts of the latter, supposing that when formed locally sodium and potassium soaps would diffuse into the blood and lymph and so escape to a very large extent; but to our surprise the sodium and potassium soaps were not nearly so soluble in tissues as they are *in vitro*—in fact they are relatively insoluble in the former location.

ON THE EXISTENCE OF A COMPOUND OF SOAPS WITH ALBUMEN.

What would seem clearly to be the explanation of this difference was discovered in conducting observations in the test tube. Doubtless the reaction which we are about to describe has been noted before, but, if so, it has failed to come to our attention. If to a weak solution of a pure soap—sodium stearate for example, though the same is true of the oleate and the palmitate—there be added dilute egg albumen, a white rather flocculent precipitate forms. The reaction is not immediate: at the temperature of the room it requires half an hour or longer before it is complete; the precipitate is a combination of soap and albumen. The compound is slightly soluble in water and less soluble in alcohol. Under the microscope the precipitate appears granular, and when the particles are treated with Sudan III they assume the

identical pinkish-yellow color of the soap granules observed in tissues undergoing calcification.[1]

It is worth noting that the precipitate becomes denser and more pronounced when carbon dioxide is passed through the solution. Acid calcium phosphate may be added to a solution of egg albumen without precipitation; but if the salt be added to a combination of weak soap solution and egg albumen, made as above, precipitation takes place rapidly and yields a dense sediment.

The Chemical Isolation of Soaps from Pus.—We have already stated that we have been able to isolate *soaps* from more than one order of calcified tissue. We were able to isolate a material soluble in absolute alcohol, insoluble in petroleum ether, and precipitable on the addition of acid calcium phosphate from a number of calcified tuberculous glands of the mesentery. On the addition of a mineral acid the fatty acid was isolated from this combination and gave the characteristic stain with Sudan III.

Particular attention is called to the fact that similar soaps may be present in pus. The cases from which the pus was examined were, it is true, of chronic nature; and no opportunity has yet come to us for the study of large quantities of pus of more acute origin. With the pus of two tubercular abscesses (one an empyema, the other a case of Pott's disease), each yielding fifty cubic centimetres, tests were made. Each quantity was extracted with warm absolute alcohol for seventy-two hours, after which the mixture was filtered and the filtrate evaporated to complete dryness. The residue consisted of fat drops mixed with a white granular substance. The residue was collected and washed with ether and petroleum ether for four days, after which no fat or fatty acid could be demonstrated in the filtrate. It was now digested with warm distilled water, and on the addition of

[1] Mr. O. R. Mabie, Phm.B., has undertaken a study of this soap-albumen compound. His studies are at the present incomplete, but he has found that solutions of albumen when mixed with known strengths of chemically pure soap solutions absorb a certain quantity of the soap. He has used albumen from different sources, and with all he has obtained similar results, although different degrees of absorption have resulted.

calcium chloride to a quantity of the watery extract a white precipitate, stainable after evaporation on a watch glass with Sudan III, was obtained. In the unstained condition the precipitate appeared as a white fine powder. Fatty acids were obtained from the residue by adding acid, and the addition of hydrochloric acid to the aqueous solution caused an opalescence in the test tube. Sodium and potassium were detected in the residue by the flame, but no calcium was found. Fatty acids could also be detected and saponified from the extract in the petroleum ether.

ON THE EXPERIMENTAL PRODUCTION OF CALCIFICATION.

In Capsules Containing Fats and Fatty Acids.—Early in the course of this research experiments were undertaken to determine the organic compounds with which calcium salts may unite. Various products obtained as pure soaps were placed in celloidin capsules prepared by the method recommended by McCrae and introduced into the peritoneal cavity of rabbits where they were left for several days. The results of these experiments led to the study of the rôle of fatty acids and soaps in the process of calcification.

Von Kossa places the normal calcium strength of the blood of rabbits as varying from 1.06 to 2.01 per cent., which may be taken as about the limits of range. In our experiments, two celloidin capsules were prepared containing one gramme of sodium stearate and one gramme of sodium palmitate respectively. These were inserted into the peritoneal cavity of a rabbit and allowed to remain there eleven days. On removal, the contents of the capsule were more or less caked and contained some serum. The contents were treated with hydrochloric acid and precipitated with ammonium oxalate, and the solution then rendered alkaline with ammonia. The precipitate was collected and the calcium determined in the ash obtained on ignition. The sodium stearate was found to have combined with the greater quantity of calcium, there being 4 per cent. of the alkaline earth present. The capsule containing the sodium palmitate was found to contain 3.8 per cent. of calcium. Hence it was found that even in the short space of eleven days a definite

conversion of the sodium and potassium soaps into those containing calcium had taken place. Other experiments were carried out in which stearic and palmitic acids were substituted for their salts. The results were similar to the first, there being a decided increase in the calcium strength over that present in the serum of the animal.

The Experimental Production of Calcareous Infiltration in the Kidney.—All cells use fat in some form in carrying out their normal physiological function, and, as has already been pointed out, a great deal of fat is brought to them as soap. When the cell is injured, the soap it would seem is not metamorphosed but remains fixed in the cell in combination with the cytoplasm. This form of union is indicated by experiments on the kidney epithelium in animals, for the main conclusions to be drawn from Litten's experiments are that the tissue cells undergoing calcification are in a degenerating state and have already lost, or are in process of losing, their vitality. He further pointed out that calcification proceeds more rapidly if along with the dead and dying cells there should remain a good circulation to supply the calcium and other materials which are eventually deposited as a permanent precipitate. As Litten examined his sections only with hæmatoxylin staining he found no evidence of fatty products laid down in the areas of his "coagulation necrosis."

The animal experiments reported by Litten were the first dealing with the question of the calcification of the renal tubules. They demonstrated that, if the renal vessels were ligated for two or more hours and then loosened, after eight or ten days crystals of lime salts were to be found in the cells of the convoluted tubules chiefly. The better the blood supply to the organ after ligation, the more quickly did the calcification proceed. The explanation offered was as follows: arrest of the blood to the kidney having caused "coagulation necrosis" of the tubular epithelium, the later advent of the calcium-containing tissue fluids brought about precipitation in the tubules of "calcium albuminate." Although this theory of Litten has been current for twenty years, it is noteworthy that the production of calcium albuminate had not been demonstrated *in vitro*.

We must, however, conclude that in the cells which are injured there is a change in the protoplasm, and of this there is adequate evidence found in the fact that the nuclei stain poorly and the cell substance appears swollen and homogeneous. The alteration of the nucleus is perhaps the chief indication that the cell has suffered grave metabolic changes. There is no evidence at this point of the process that the cell substance is diminished in amount—indeed, on the contrary, the actual amount of cell material appears increased. This is in keeping with the recent observations which point to the conclusion that fat appearing in the cell is not the product of cytoplasmic decomposition, but is absorbed from without. The view we wish to emphasize is that in the degenerating cells absorption of material from without still continues whether this material be albuminous or fatty. But owing to nuclear disturbance, or more broadly to lowered metabolic activity, the substances so absorbed cannot be used up; they no longer become combined into the cell cytoplasm or bioplasm proper, and remaining in an uncombined state may now become subject to various pathological changes.

Neuberger has shown that a number of chemical irritants produce the same effect on the kidney as ligating the renal vessels, and that they lead to calcification of the same sets of tubules. We repeated Neuberger's experiments, using the salts of the heavier metals, mercury, copper, lead, etc., with the result of producing calcareous degeneration in the convoluted tubules of the cortex of the kidney. The rabbits were inoculated subcutaneously with solutions of the soluble salts of these metals and then killed after ten days. A small portion of the kidney was removed for microscopical purposes. Frozen sections were stained with Sudan III and silver nitrate and both gave satisfactory pictures. In consecutive sections it could be shown that the degenerated tubules which reacted for the fatty substances with Sudan III also reacted with silver nitrate; and, moreover, that when a section was treated first with Sudan III and after staining was subjected to silver-nitrate solution, all the Sudan-III-staining tubules were covered over by the intense black stain of the silver salt. That is, the identical tubules which

were shown to contain a fatty-staining substance also contained calcium salt (vide Figs. 1 and 2, Plate xxx). The calcium could be wholly removed from the tissue by the use of dilute acids without affecting its fatty-staining qualities. If frozen sections were treated first with seventy per cent. alcohol and later with petroleum ether, it was still possible to find the tubules picked out with Sudan III and silver nitrate. Finally it has been possible to extract from the cortex of the kidney, potassium, sodium, and calcium soaps. These results indicate that the formation of a calcium soap is one stage in the process of calcification in the kidney; that, degeneration and death of the cells being effected, the cells are not able to utilize the potassium and sodium soap brought by the blood, and hence these are precipitated in combination with albumens, and later the calcium from the tissue fluid and blood is fixed in an insoluble state by this compound. While we agree with Litten, Neuberger, von Kossa, and others, that necrobiosis, improperly referred to by them as a "coagulation necrosis," occurs, we look upon this as only one stage in the whole process of calcification.

Von Werra believes that such degenerated cells of the kidney may return to a normal condition. We can hardly accept this view, as calcareous degeneration of cells is the last stage in a pathological process in which the cells and nuclei are disintegrated. Hence it is improbable that they can regain their vitality.

Tartarini-Galleani claims to have studied the kidneys of rabbits after injecting bichloride of mercury into the substance of the organ. He states that the lesion produced is a tissue destruction followed by fibrinous coagulation, which after the ninth day becomes impregnated with calcium salts. The calcification in the kidney remains circumscribed to the area of the organ directly affected by the injection, and he holds that it is neither preceded nor accompanied by the phenomenon of fatty change. We have repeated his experiments in two rabbits and in each the result was different from that described by him.

Sections of the inoculated kidney treated with Sudan III showed the cells of the convoluted tubules to be swollen and

filled with fatty granules. The tubules were well outlined from the surrounding tissue by reason of the stained fat in the cell contents. The glomeruli and the interstitial tissue appeared unchanged, while the arterioles of the cortex showed Sudan-III-stained granules in their walls. These granules were distributed more loosely than was seen in the epithelium. The affected convoluted tubules presented collections of irregular granules staining not so intensely as neutral fat, but rather a brownish-yellow. The tubules in the pyramids showed a striking difference. While they were less affected than in the cortex the condition of the fat in them differed from that of the convoluted tubules. The individual cells showed no change in size or shape, and the nuclei retained their staining power. However, the Sudan III picked out numerous fine globules situated towards the inner margins of the cells—that is, towards the lumen and away from the nucleus. In some cases it appeared that the fatty globules were in the process of being extruded into the lumen. The Sudan III granulation noted in the straight tubules was of the character of neutral fat. No calcium deposit could be demonstrated in the straight collecting tubules, while in the cortex the convoluted tubules which showed the Sudan III granulations reacted to silver nitrate in the manner of calcium salts.

Résumé.—Our studies upon experimental calcification in the kidney confirm and extend the observations made upon calcareous changes in human tissues. If the tubules become the seat of the calcareous deposit it is possible to recognize the following stages, viz:

(1) A stage of cell degeneration characterized by swelling of the cell substance and diminution of the nuclear chromatin, the cells becoming swollen and homogeneous;

(2) A later stage in which fat appears in the cells, apparently having arisen by absorption.

(3) A final stage in which calcareous salts appear in the cells accompanied by soaps. An intimate relation between cytoplasmic degeneration and the formation of insoluble soaps would seem to exist. Judging from observations made in test tubes,

the relatively insoluble soaps are compounds between soap and certain albuminous matters liberated from the cytoplasm. The calcium from the tissue fluids and the blood becomes fixed by the albumen-soap compound.

Experimental Calcification of Muscle Fibres.—That muscle fibres may become the seat of calcareous deposits is well known. We inoculated a weak solution of bichloride of mercury into the quadriceps muscle of a rabbit, killing the animal at the end of eight days. On examination the muscle at the site of the inoculation showed extensive degeneration and softening. Individual fibres were swollen and in some places the sarcolemma was broken. Other swollen fibres showed band-like constriction. Still other muscle fibres were completely broken down and invaded by numerous polymorphonuclear leucocytes.

The degenerated muscle fibres proved an interesting study. A fine granulation not found in healthy fibres, and staining in Sudan III, occurred in their substance, besides which leucocytes loaded with fat globules invaded the degenerating fibres and lay in the interstitial tissue. The fatty-staining material within the leucocytes appeared in the form of large and small granules, but these did not coalesce to form large fat globules as is seen in adipose tissue.

The question arises as to whether the leucocytes are bringing the fat to the injured tissue or taking it away. We are inclined to the latter view, for among the degenerated fibres were some in which the sarcolemma was intact and which were free from leucocytic infiltration, but which nevertheless contained granules staining with Sudan III. Moreover, the leucocytes at the margin of the injured tissue contained no fatty-staining material.

We think it probable that this process is not unlike that described in the kidney, namely, a state of lowered vitality of the individual cells through which, being no longer able to deal with the fatty material brought to them in solution, they form with it a relatively insoluble compound in the cytoplasm of the degenerating cells.

Experimental Fat Necrosis.—In order to investigate further the changes which fatty products undergo in the body, we pro-

duced fat necrosis in a rabbit by the intraperitoneal inoculation of fresh extract of pancreas. The injection was repeated on the second day and the animal killed on the fifth day. At the post-mortem a few tags of fat necrosis about the size of a pea were found scattered in the mesentery and the posterior abdominal wall. Frozen sections of these showed that the fat did not exist in the large distended fat cells but that the globules were replaced by numerous fine crystalline particles staining with Sudan III. On treating the sections with silver nitrate, the subperitoneal margins of the fat necrosis showed the presence of a slight amount of calcium salts appearing like a fine dust-like deposit. All the areas of fat necrosis did not show the presence of calcium with silver nitrate

As already stated, Langerhans showed in 1890 that calcium salts appear in the areas of fat necrosis. He describes the process as one in which the fat is converted by ferment action into fatty acid. The calcium salts of the blood then become attracted to the fatty-acid moiety and form a calcium soap.

ON THE SOURCE OF THE FAT IN THE DEGENERATED CELLS.

Before concluding, it is, we think, essential to indicate our views regarding the source of the fat present thus in all cells that undergo calcification and essential for the due development of the process. To discuss the matter adequately would demand that the numerous recent studies upon fatty degeneration be passed in review: such full discussion would carry us too far afield. It must suffice that we call to mind the reasonable deductions from the work of recent observers which appear to bear immediately upon our present problem.

We may, I think, in the first place safely accept that fats are absorbed by the cell from the surrounding medium in the form of diffusible soaps. Taken up by the cell these soaps may either be reconverted into neutral fats and stored as such in the cytoplasm, or may undergo assimilation proper, becoming part and parcel of the cell substance. That such assimilation does occur with associated loss of individuality on the part of the fats is,

we hold, demonstrated by sundry recent observations. Thus Rosenfeld and others have shown that kidneys presenting no fat recognizable by microchemical means will, nevertheless, yield upon careful analysis as much as fifteen per cent. of fats; and Doremeyer, that mere extraction by ether will not liberate the greater part of tissue fats: the cells have to be digested with pepsin before all can be isolated; a clear indication that the fats have previously been in combination with proteid substances in the cells.

If, then, these possibilities be accepted, it follows that the appearance of fats and soaps in a degenerating cell may be from at least two sources, namely:

(1) The absorption of soaps from the circulating lymph and blood, the cell still retaining the power of splitting off the fat, but being unable to utilize this in metabolism, so that it becomes stored up in a non-diffusible form.

(2) Disintegration of the cell substances so that the fats become liberated from their combination with the cytoplasm and appear in the form of discrete particles, or globules, of neutral fat, which now reveal their presence by reacting with Sudan III.

A third possibility has to be kept in mind, namely, that soaps may be absorbed from the blood and lymph by cells with vitality so depressed that no conversion takes place of the absorbed soap into neutral fat, but combining with the disintegrated albuminous molecules this forms a relatively insoluble soap-albumen compound.

Into the controversy regarding the formation of fat by the decomposition of the albuminous molecule I will not enter, save by suggesting that the opposing views may possibly be harmonized through the demonstration here afforded, that albumens form a combination with soaps. The increase in fats determined in tissue autolysis by Waldvogel, Hildesheim and Leathes, Landsteiner and Mucha, and others is, it may be, to be explained, not as due to the appearance of a decomposition product of the albumen molecule proper, but as brought about by dissociation of this albumen-soap compound.

The time is not ripe for, neither do our personal observations

up to the present permit, any discussion of the part played by cell ferments in any of these processes.

As to which of the above alternatives—absorption or dissociation—is the more frequently in action as a precursor of calcification, we would point out that when the cell becomes distended with fatty globules (and this is common in atheromatous areas) we must conclude that absorption from without is in the main responsible for the condition.

CONCLUSIONS.

It will be seen from the above that we have studied the conditions associated with the deposit of calcareous salts: (1) in connection with normal and pathological ossification, and (2) in pathological calcification as exhibited in (a) atheroma of the vessels; (b) calcification of caseating tubercular lesions; (c) calcification of inflammatory new growth, and (d) degenerating tumors; and we have induced experimentally deposits of calcareous salts in the lower animals: (a) within celloidin capsules containing fats and soaps; (b) in the kidney, and (c) in connection with fat necrosis.

I. We have found that bone formation and pathological calcareous infiltration are wholly distinct processes. In the former there is no evidence of associated fatty change, and the cells associated with the process of deposition of calcium are functionally active. In the latter there is an antecedent fatty change in the affected areas, and the cells involved present constant evidences of degeneration. The view that would seem to account best for the changes observed in the latter case is that with lowered vitality the cells are unable to utilize the products brought to them by the blood, or which they continue to absorb, so that the normal series of decompositions associated with their metabolism fails to take place and hence they interact among themselves in the cytoplasm with the result that insoluble compounds replace soluble ones.

II. Besides the fact that calcification is always preceded by fatty change within the cells, another fact should be emphasized,

namely: that combination of the fats present with calcium salts to form calcium soaps tends to occur. The stages immediately preceding these are difficult to follow with anything approaching certainty, perhaps because the earlier stages vary under different conditions. In fat necrosis, for instance, the cells affected are normally storehouses for neutral fats, and as long as they remain healthy neutral fats alone are present in them. When they are subjected to the action of the pancreatic juice with its fat-splitting ferment the cells are killed and coincidently the neutral fats are decomposed, fatty acids being deposited. The fatty acids now slowly combine with the calcium salts. In degenerating lipomata the process would seem to be similar.

But in other cases the cells are not obviously fat-containing in the normal state; nevertheless prior to calcification they undergo so-called fatty degeneration, which is really a form of cell degeneration accompanied by fat infiltration. As regards the source of the cell fats in general we may safely accept:

1. That fats are transported in the blood as diffusible soaps.
2. That taken up by the cells these soaps may either—
   (a) Be reconverted into neutral fats and become stored in the cytoplasm as such, or
   (b) undergo assimilation proper, becoming part and parcel of the cell substance, in which case they are not recognizable by the ordinary microchemical tests.
3. If these two possibilities be accepted it follows that the appearance of fats and soaps in the degenerating cell may be due to either—
   (a) Absorption or infiltration of soaps from the surrounding medium, the degenerating cell retaining the power of splitting off the fat but being unable to utilize this in metabolism.
   (b) Cytoplasmic disintegration with dissociation of the soap-albumen combination or, more broadly, liberation of the fats from their combination with the cytoplasm.

The appearances seen in the cells of atheromatous areas indicate that the first of these does occur.

III. In areas undergoing calcareous infiltration we have demonstrated the presence of soaps, and this often in such quantities that they can be isolated and estimated by gross chemical methods. By microchemical methods also we have been able to show that after removing all the neutral fats and fatty acids by petroleum ether there remains behind a substance giving with Sudan III the reaction we associate with the presence of soap. And experimentally we have produced these soaps within the organism, more particularly by placing capsules containing fats and fatty acids within the tissues and after several days finding that the capsules contain calcium soaps and possess a calcium content far in excess of that of the normal blood and lymph.

IV. While these are the facts, certain of the details of this reaction demand elucidation. The existence of sodium and it may be potassium soaps in the degenerated cells is comprehensible if we accept that these are present in the circulating lymph and simply undergoing absorption. But even then, as these are diffusible substances how is it to be explained that they become stored up in these particular areas?

We have found that, as a matter of fact, in regions which give the reaction for soaps, but which give no reaction for calcium (which therefore presumably contain at most amounts of the insoluble calcium soap too small to need consideration), the ordinary solvents for potassium and sodium soaps do not forthwith remove the stainable material; they are relatively insoluble.

The reason for this insolubility is suggested by the observations made in the test tube, that soap solutions mixed with solutions of white of egg or blood serum form a precipitate of combined soap and albumen,which likewise is insoluble in water and alcohol. The indications are therefore that in cells undergoing degeneration, with degeneration of the cytoplasm, certain albuminous molecules unite with the soaps present to form relatively insoluble soap-albuminate.

V. With regard to calcium soaps, these are also present and in certain stages appear to be the dominating form in the affected tissues. Two questions suggest themselves, viz.: what is the source of calcium, and what is the process by which they become formed?

As to the source, the amount present in well-marked calcification is far in excess of the normal calcium contents of the affected tissue. If in the kidneys of experimental calcification three hundred times as much calcium may be present as in the normal kidney (von Kossa), the calcium must be conveyed to the part by the blood and lymph, and that this is so is demonstrated, as we have pointed out, by the distribution of the infiltration in solid organs, that like ovarian fibroids have undergone necrosis, in which the earliest deposits are superficial.

As to the process, there are three possibilities:

1. That sodium and potassium soaps and soap albuminates are first formed and that interaction occurs between them and the diffused calcium salts from the lymph, the less soluble-calcium replacing the sodium and potassium.

2. That under certain conditions the calcium salts act directly on the neutral fats present in the degenerating cells.

3. That the neutral fats are first broken down into fatty acids and that these react with the calcium salts to form the soaps.

We are assured that the first process occurs and that because in the boundary zone of areas of calcification we can detect soapy particles devoid of calcium, identical in position and arrangement with the particles more deeply placed which give the calcium reactions. But this is not the only reaction. In case of fat necrosis we see clearly that the third process is in evidence. And we are far from being convinced that the second does not also obtain. We have been impressed by the large accumulation of neutral fats in the cells in cases of early atheroma and the absence at any stage of the process of recognizable fatty acid. While soaps, it is true, are compounds of fatty acids with alkalies, it is recognized in ordinary domestic life that they can be formed by the direct action of strong lye upon ordinary fats, and this even in the cold. It is quite possible therefore that there occurs a similar direct process in the organism.

The point is worth noting, however, that this does not occur in healthy cells the seat of fatty infiltration. We therefore leave this an open question, only laying down that, as indicated by

the hyalin albuminous matrix left when calcium salts are dissolved out of an area of calcification, there must exist a calcium soap- or fat-albuminate similar to the potassium and sodium soap-albuminates already mentioned—such an albuminate as we can form with calcium soaps in the test tube.

VI. In old areas of calcification soaps are largely if not entirely wanting, although these are to be detected at the periphery, when the process is still advancing. The reactions given by these older areas are almost entirely those of calcium phosphate, though some calcium carbonate is at times to be made out.

This seems surely to indicate that the final stage in calcification is an interaction between the calcium soap-albuminates and substances containing phosphoric and carbonic acids. Such substances, it is needless to say, are present in considerable amounts in the lymph and blood. We must conclude that the acid sodium phosphates of the lymph act on the calcium soap, the highly insoluble calcium phosphates being formed (plus the albuminous moiety of the original compound) and diffusible sodium soap being liberated, while similarly alkaline carbonates form calcium carbonate and liberate sodium and potassium soaps. Calcium phosphate and calcium carbonate thus become the insoluble earthy salts of old crystalline areas of calcification.

VII. As already stated very little soap is to be found in these old areas. It is possibly worth suggestion that the soaps liberated in this last reaction, as they diffuse out, again react with diffusible calcium salts and form calcium soaps which once more react with the alkaline salts to produce the phosphates and carbonates; that, in short, they have a katalytic action. Certain it is that old calcareous areas are extraordinarily dense, and have a coarse crystalline structure, wholly at variance with the finely granular appearance of the more recent areas, and these large crystalline masses, it would seem, can only be formed by successive deposition of new material and eventual fusion, as the interspaces become filled in between the original masses.

In conclusion I wish to thank Professor R. F. Ruttan of McGill University and Mr. J. R. Roebuck, B.A., for their kind assistance in carrying out some of the chemical analyses, and Drs. M. E.

Abbott and F. A. J. Lockhart for specimens of calcareous degeneration of tumors.

BIBLIOGRAPHY.

Arnold, *Münchener med. Wochenschrift*, 1902, xlix, 1945; *Virchow's Archiv*, 1903, clxxi, 197.

Beebe, *American Journ. of Physiology*, 1904, xii, 167.

Beebe and Buxton, *American Journ. of Physiology*, 1904, xii, 466.

Beneke, *Ziegler's Beiträge*, 1897, xxii, 343; *Virchow's Archiv*, 1900, clxi, 194.

Cohnstein and Michaels, *Pflüger's Archiv*, 1897, lxv, 473.

Dormeyer, *Pflüger's Archiv*, 1897, lxv, 90.

Fischler, *Virchow's Archiv*, 1902, clxx, 100; *Virchow's Archiv*, 1903, clxxiv, 338; *Centralblatt f. Pathologie*, 1904, xv, 913.

Gierke, *Inaugural Dissertation*, Berlin, 1902.

Hanriot, *Archiv de physiologie*, 1898, x, 797.

Hildesheim and Leathes, *Jour. of Physiology* (Proceed. of Physiolog. Soc.) 1904, xxxi, 1.

Irvine and Sims Woodhead, *Laboratory Report, Royal College Physicians, Edinburgh*, 1, 1889; 11, 1890.

Jaeckle, *Hoppe-Seyler's Zeit. f. Physiol. Chemie*, 1902, xxxvi, 53.

v. Kossa, *Ziegler's Beiträge*, 1901, xxix, 163.

Kraus, *Verhandlungen d. deutsche path. Gesellschaft*, 1904, 37.

Kyber, *Virchow's Archiv*, 1880, lxxxi, 436.

Landsteiner and Mucha, *Centralblatt f. Path.*, 1904, xv, 752.

Langerhans, *Virchow's Archiv*, 1890, cxxii, 252.

Litten, *Zeitschr. f. klin. Med.*, 1879, i, 131; *Virchow's Archiv*, 1881, lxxxvi, 508.

McCrae, *Jour. of Experimental Medicine*, 1901, v, 635.

Marcet, *Proceedings of Royal Society*, 1858, ix, 306.

Munk, *Cent. f. d. med. Wissensch.*, 1889, 514.

Neuberger, *Archiv f. exp. Path. u. Pharmacol.*, 1890, xxvii, 39.

Pfahler, *New York Medical Journal*, 1902, i, 270.

Ramond, *Ref. Bulletin d. l'Inst. Pasteur*, 1904, ii, 726; *Archiv d. méd. exp. et anat. path.*, 1904, xvi, 655.

Ribbert, *Verhandlungen d. deutsche path. Gesellschaft*, 1904, vi, 48.

Rosenfeld, *Verhandlungen d. deutsche path. Gesellschaft*, 1904, vi, 73; *Berlin. klin. Wochenschrift*, 1904, xli, 34.

Schultz, *Pflüger's Archiv*, 1897, lxv, 299.

Schultz and Schwalbach, *Pflüger's Archiv*, 1894, lv, 231.

Schwalbe, *Verhandlungen d. deutsche path. Gesellschaft*, 1904, 71.

Tartarini-Galleani, *Lo Sperimentale*, 1904, lviii, 371; *Ref. Medical Chronicle*, 1904, xli, 116.

Traina, *Ziegler's Beiträge*, 1904, xxxv, 1.

Virchow, *Virchow's Archiv*, 1856, ix, 618.

Waldvogel, *Virchow's Archiv*, 1904, clxxvii, 1.

v. Werra, *Virchow's Archiv*, 1882, lxxxviii, 197.

FIG. 1.

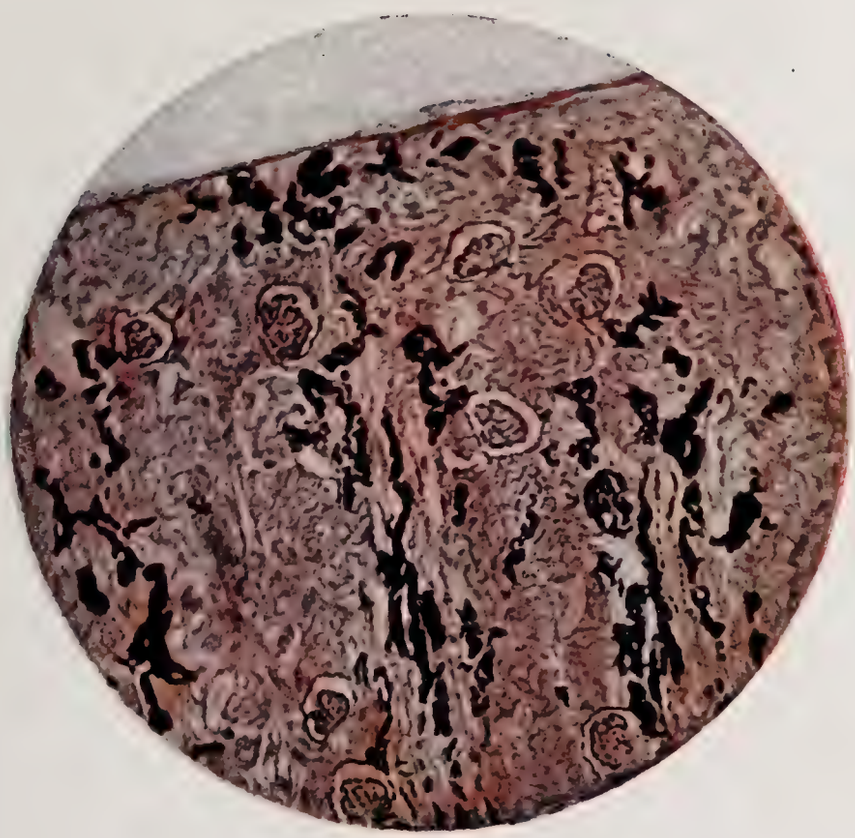


FIG. 2.

FIG. 3.

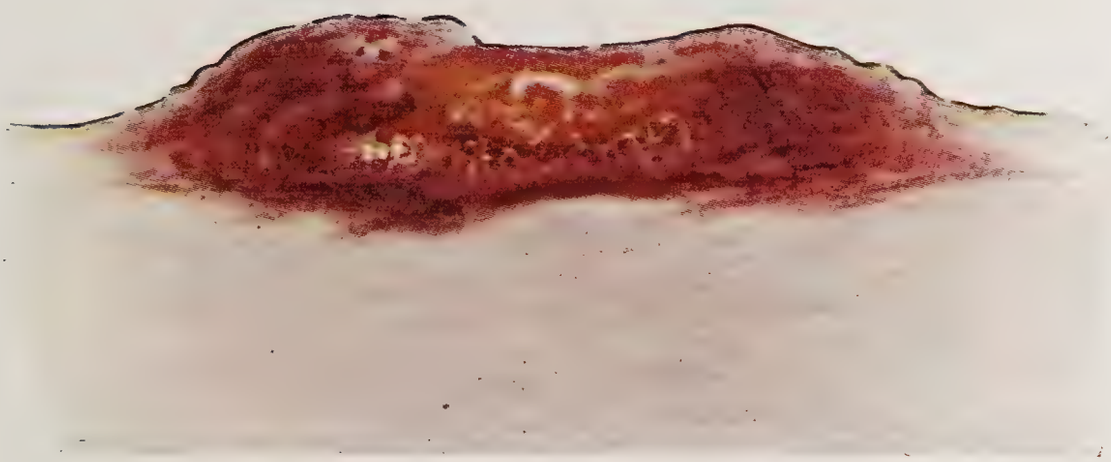


FIG. 4.

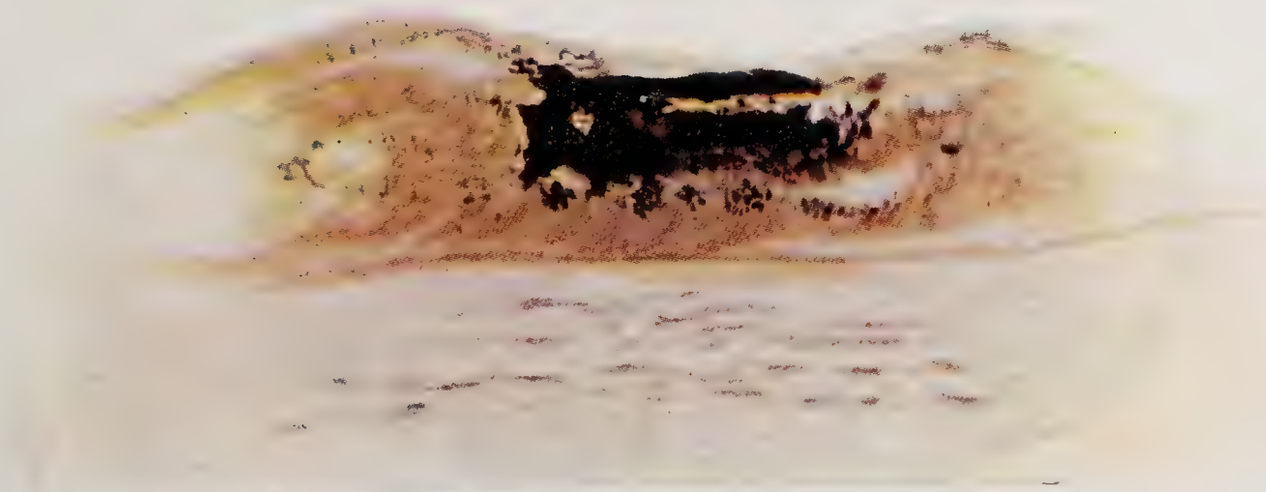


FIG. 5.

DESCRIPTION OF PLATES.

Plate XXX.

Fig. 1.—Kidney from rabbit which died eight days after inoculation with corrosive sublimate. Frozen section stained with Sudan III showing the fatty condition of certain convoluted tubules.

Fig. 2.—Section from the same kidney as Fig. 1, stained with silver nitrate followed by weak saffranin. Similar convoluted tubules picked out by the silver deposit as seen in Fig. 1 with Sudan III.

Plate XXXI.

Fig. 3.—Aorta with large fat masses towards the lumen with similar lighter staining particles near the boundary zone between the intima and media. In the media the blackened deposit representing calcium salts is very intense and is arranged in parallel rows. These rows with higher magnification are seen to be composed of numerous small crystals. Among these rows of calcium salts are also seen the yellowish granules of Sudan III staining.

Fig. 4.—Section of aorta stained with Sudan III. The fatty plaque shows a lighter stained part in its middle, representing the area of soap formation and calcium deposit.

Fig. 5.—Same aortic plaque as seen in Fig. 4, treated with silver nitrate solution. The area which with Sudan III stained a light yellow is now represented as the black deposit, the site of the calcium salts.

STUDIES UPON CALCAREOUS DEGENERATION.

STUDIES UPON CALCAREOUS DEGENERATION.

II. THE STAINING OF FATTY ACIDS AND SOAPS IN THE TISSUES BY FISCHLER'S METHOD, AND A MODIFICATION OF THE SAME.

III. CALCIFICATIONS OF THE AORTA IN RABBITS AFTER THE INOCULATION OF ADRENALIN.

IV. CALCIFICATIONS OF THE MEDIA IN ARTERIES OF THE ELASTIC TISSUE TYPE.

By OSKAR KLOTZ.

Resident Pathologist, Royal Victoria Hospital; Late Governors' Fellow in Pathology, McGill University, Montreal.

(From the Pathological Institute, Bonn; Professor Ribbert, Director.[1])

II. THE STAINING OF FATTY ACIDS AND SOAPS IN THE TISSUES.

Quite recently Fischer[2] has given us a method, a modification of Benda's copper acetate process, of demonstrating the presence of fatty acids in the tissues, either in the free state or in combination with bases in the form of soaps. By this method the fatty acid compounds are converted into the insoluble copper soaps, which in the presence of hæmatoxylin form a varnish-like substance insoluble in weak acids. The chemistry of the combinations is not as yet fully determined, particularly in regard to the reactions with oleic acid. The process, however, is a very precise one, if the tissue be placed in the copper acetate solution immediately after it is removed from the body, when it is found that the soluble and insoluble soaps, along with the fatty acid in the free state, are picked out very clearly. If, however, as Fischer points out, the tissue be placed in water or an aqueous fixing fluid for some time preceding the treatment with copper acetate, a loss (more or less) of the soluble soaps results.

[1]This study was aided by a grant from the Rockefeller Institute for Medical Research.

[2]Fischer, *Centralbl., f. Allgem. Pathol.*, 1904, xv, 913.

I say more or less, for, as I have previously pointed out,[3] what constitute soluble soaps in the test tube are not necessarily such in the body.

The method is determinative only for the fatty acid radical, so that the relative quantities of soaps and the free fatty acids cannot be determined. It is true that Fischer advises fixing one portion of the tissue under examination in a formalin solution, while another portion is fixed in a saturated solution of calcium salicylate in 10 per cent. formalin. Both bits of tissue are then treated with the copper acetate solution and the other two fluids in the usual manner. The tissue fixed in the latter solution will demonstrate the combined presence of the free fatty acids and the soaps, while in the former solution some of the soaps have been dissolved out in the fixing fluid, and it was thought that only the free fatty acid radical would remain. However, the calcium soaps, as Langerhans demonstrated them in fat necrosis, and further as have been shown to be present in all pathological processes of calcification, are also almost insoluble formalin in solution, as are also the albuminous compounds of soaps, so that in the formalin-hardened specimen I demonstrated more than the free fatty acid.

The method, however, is very useful in determining the fatty acid radical, free or combined, in microscopic sections, particularly where calcification is progressing in the tissue. The process further has supported my views regarding the process of pathological calcification, that this constantly occurs through the intervention of fatty acids. It is to be noted, as I have pointed out, that the advanced portions of calcified areas no longer contain fatty acids, for the calcium has in these areas formed a more stable compound with carbonic and phosphoric acids.

A modification of Fischer's method of staining the fatty acids brings this still more clearly to light in such sections. If instead of his second solution, which is a saturated solution of hæmatoxylin in absolute alcohol, I substitute a 60 per cent. saturated alcoholic solution of hæmatoxylin, I avoid disturbing the neutral fats present in the degenerated tissues about

[3] Klotz, *American Journal of Physiology*, 1905, xiii, Supp. No. 1, 21.

calcified areas, and thus allow the use of Sudan III or Scharlach R. to stain these.

The process then becomes in short:

1. Fix the tissue and precipitate the fatty acid radical by treating the sections for 1 to 24 hours in the following solution:

Chromalum, 2.5 grams
Formalin, 4%, 100 c.c.
Dissolve by boiling.
While cooling add
Acetic acid, 5 c.c.
and then
Neutral copper acetate (powdered), 5 grams.

2. Thoroughly wash in water.

3. Cut on the freezing microtome.

4. Stain sections in a saturated solution of hæmatoxylin in 60% alcohol for six hours.

5. Wash sections in water and then treat with the following fluid (Weigert's decolorizing fluid) until the tissue becomes a light brown, while the sites of the fatty acid radical remain black.

Potassium ferricyanide,	2.5	grams
Borax,	2.0	"
Water, distilled,	100.0	c. c.

The sections can then be mounted in Canada balsam.

Blue-black deposits are to be noted in areas where larger quantities of calcium soaps are found.

It is to be remembered that in this method only the copper which is deposited along with the fatty acid stains, and that the structure of the fatty acid compounds as they exist in the tissues is lost. However, this is a minor point, and I am able to determine the smallest particles of the free or combined fatty acid moiety. Now if I wish further to obtain the relation of the fatty acids to the remaining fatty degeneration in the tissue I can stain the sections with Sudan III or Scharlach R.

This combined method of demonstrating the fatty acids and their salts along with the neutral fats is of the greatest service in obtaining a conception of the process of calcification. Thus in the centre of a calcified area the blue-black crystals of the inorganic calcium salts are noted; these are then bordered by an intense black area having outrunners into the surrounding tissue, representing the free fatty acids, the calcium and other soaps; and beyond this area again are to be seen the Sudan stained neu-

tral fats in the degenerating tissues. The tissues which contain fatty acids or their calcium salts are necrotic, and contain no living cells.

This combination of Fischer's fatty acid stain with Sudan III I have used in demonstrating the relation of the fatty acids and their salts to the fatty degeneration in calcified tumors, calcification of the arteries, and phleboliths, and have found all to be of the same character. If in other sections the neutral fats are stained with Sudan III while the calcium salts are blackened with silver nitrate (v. Kossa's method) I can compare the quantity of calcium salts to the fatty acid moiety and roughly determine the amount of calcium soaps present in the tissue. As the process of staining the fatty acids and that of demonstrating the calcium salts both yield black precipitates, I cannot utilize the combined method in the same section. However, as the three staining processes mentioned demonstrate in frozen sections the various stages which calcareous degeneration undergoes, I can follow step by step the stage of fatty degeneration, the production of fatty acids, and the calcium soap formation in all tissues in which pathological calcification is progressing. I have found it advisable to decalcify the tissues in which the fatty acid radical is to be demonstrated, to avoid the confusion with the darkened calcium granules in the finished sections. This is done after applying the copper acetate solution.

CALCIFICATION OF THE AORTA IN RABBITS AFTER THE INOCULATION OF ADRENALIN.

In a recent number of *Virchow's Archiv*, Scheidemandel[4] discusses the changes occurring in the vessels in rabbits, after the inoculation of adrenalin. Without entering into my own experiments with adrenalin, or touching upon the similarities which these vascular changes bear to arteriosclerosis, it is proper to criticise some of his conclusions which do not conform with the results given by the methods described by me in previous communications.

In his sections of the aorta he finds, when stained with hæma-

[4] *Virchow's Archiv*, 1905, clxxxi, 363.

toxylin and eosin, that between the strands of elastic fibres blue staining granules are deposited in the muscle cells in which the nuclei are still present. In the heavier deposits the whole cells are obliterated and the calcium salts form dark staining bands. The elastic fibres in the areas of calcification, he points out, show a narrowing of their lamellæ, and their staining characters are changed so that they appear granular. In some places he noted tears of the elastic elements. He did not observe any fatty degeneration, nor was he able to determine a calcification of the elastic fibres. He further claims that the elastic fibres are the first to show the degenerative changes in the vessel wall.

In my experience, the deposit of calcium salts within living cells, to the extent that they are stainable with hæmatoxylin, is a very questionable occurrence. The process of calcification is one which does not permit of this, but what I do find, is that in degenerated areas there still exist cells which have not undergone the general necrosis, and in these the nuclei are yet to be seen while the broken down cells about them become calcified. Where living cells exist in calcified areas they do not contain calcium salts to the extent that they will stain with hæmatoxylin. For the detection of finer quantities of calcium, hæmatoxylin stain gives an inadequate reaction, silver nitrate being a more sensitive indicator.

As I have previously pointed out, the process of calcification is preceded by a fatty change, which is followed by a conversion of these neutral fats in the necrotic areas into the fatty acids, and with them the calcium from the blood and lymph forms insoluble soaps.

I have in my possession calcified aortas from rabbits, produced by the inoculation of adrenalin, which present the various stages of the process. The early changes, as has been described by Fischer, are degeneration of muscle fibres. These are shown in my specimens, when stained with Sudan III and hæmatoxlyn, to be fatty degenerations, the fine fat granules lying about the nuclei of the muscle cells. In the more advanced stages the fat granules occupy the greater part of the cells, and the

nuclei show degenerative changes. In this condition of the muscle cells the elastic fibres are packed more closely together (Fischer), which leads to a thinning of the vessel wall. If the calcified vessels be stained with Sudan III, I find that the area of calcification is usually the site of a fat-staining deposit—the calcium soap. I say usually, for in the advanced conditions the calcium soaps have been converted into the calcium carbonate and phosphate. However, the simple application of Sudan III to a calcified vessel may show little or no fatty staining material, although such may be present. If in this case I decalcify the vessel before applying the stain I can then demonstrate the fatty substance. I take it that in such cases the calcium forms a combination with both the fatty acids and the carbonate group, thus, $Ca_n \begin{cases} \text{Fatty acid} \\ Co^3 \end{cases}$, and that the combination does not stain with Sudan III until I free the fatty acid from the carbonate. When, however, the calcium forms a combination with the fatty acid moiety alone, as I have noted it in several specimens, then I can demonstrate this fatty acid moiety by means of Sudan III. Hence the calcium, deposited in vessels, exists in the combinations, (a) the pure calcium soap, Ca-Fatty acid, (b) the calcium compound with a fatty acid and a carbonate-radical, $Ca_n \begin{cases} \text{Fatty acid} \\ Co_3 \end{cases}$, which stains with Sudan III only after decalcifying, and (c) the calcium carbonate, $CaCO_3$, which under no conditions stains with this stain. (Phosphoric acid may replace the carbonate in each of the above compounds.)

That such a fatty acid moiety exists, can also be demonstrated by Fischer's method of staining the fatty acid radical. But similar to the Sudan stain, if the calcium is in combination with both the fatty acid moiety and the carbonate radical, the specimen must primarily be decalcified before applying the reagents. In these specimens the fatty acids and the neutral fats can be demonstrated in the same section by means of the modification of Fischer's stain that I have previously described.

That calcification of the elastic fibres does occur in vessels is not a new subject (Jores, Matusewicz), and I have several

examples of the process in human arteries. In the arteriers of rabbits where the elastic fibres are very slender it is impossible to differentiate by means of the hæmatoxylin stain the calcareous granules occurring in the debris of the cells from those which occur in the elastic fibres. It is true in the greater number of aortas showing calcification the elastic fibres are the last to be affected, and they can be distinguished as the wavy refractile lines passing through the dark blue mass. However, using silver nitrate in a weak acetic acid solution, to detect calcium deposits, I find, occasionally, the primary fatty degeneration of the elastic fibres, followed by the deposit of calcium salts. By this method the calcium deposit forms a network of black granules about the muscle bundles. It is the exception to find primary fatty or calcareous degeneration in the elastic fibres, and I must consider the degeneration of the muscle bundles as the rule.

What I would particularly emphasize is that the primary degeneration leading to the subsequent calcification in the aortas of experimental animals, is found in the muscle tissue, and that it is only later that the elastic fibres are changed. A fatty degeneration is to be noted in the muscle fibres long before any macroscopic change is seen in the vessel. The sections of such vessels when stained with Fischer's *fuchselin-scharlach* show that the fatty droplets all lie between the strands of elastic fibres.

Scheidemandel points out that in the calcified aortas of rabbits the elastic lamellæ no longer take the elastic stain characteristically, and that the fibres are granular with light or unstained spaces between the granules. These unstained parts are, as he notes, areas of degeneration, and I would further point out that with the use of *fuchselin-scharlach* these spaces are seen to be the sites of fatty degeneration of the elastic fibres, that is, the degenerations occurring in the elastic fibres are also of a fatty nature, and the changes being similar to those found in the muscle cells these fibres may become the site of calcium deposits.

Of rupture of the elastic fibres, in the vessel wall, I have never been able to convince myself, and must say that those that I have seen, outside of complete tears of one or more tunics in

dissecting aneurysms, were artefacts. It is quite evident that, with a degeneration of the muscle fibres, if a rupture of the elastic elements should occur, the intervening space must be filled with blood or coagulated lymph. This is never seen in these specimens. What does happen is that more or less extensive degeneration of the muscle and elastic fibres is found in which these elements no longer take their characteristic stain. It is true that spaces with the ends of the elastic fibres jutting into them from the side are to be seen and that these appear like ruptures of the tissue, but it can be shown with Sudan III and often with the elastic stain that the wavy outlines of the former elastic fibres still stretch through the degenerated area. In human vessels, where degenerations are rarely produced as rapidly as in experimental animals, the new-formed connective tissue fills in the necrotic areas almost as fast as they develop. As Scheidemandel points out, the calcified vessels in rabbits are converted into rigid tubes and no doubt it is in the handling of these fragile sections that the apparent *intra vitam* ruptures in the inner part of the vessel wall result.

In short, the degenerations occurring in the aortas of rabbits inoculated with adrenalin are found chiefly in the muscle cells of the media, and are primarily of a fatty nature, and the inequalities in the staining reaction in the elastic fibres are due also to fatty degeneration of those elements. The calcareous deposits are consequent to the fatty degeneration in that the neutral fats in the degenerated area are converted into fatty acids, which then combine with calcium. The elastic fibres are rarely primarily affected in the degenerative changes, but when they are they may become the primary site of the deposit of calcium. The apparent ruptures of the elastic fibres are either areas of degeneration through which the degenerated fibres still pass, or are artefacts (excluding dissecting aneurysms).

I have to thank Dr. B. Fischer for some of the calcified aortas of rabbits, on which the studies were carried out. A more complete paper dealing with the degenerative processes found in human arteries is to follow shortly.

CALCIFICATION OF THE MEDIA IN ARTERIES OF THE ELASTIC TISSUE TYPE.

Calcification occurring in the walls of arteries, as one of the degenerative changes in arteriosclerosis, has been extensively described. Particularly well known are the forms of calcification that are found in the intima alone, and which are usually secondary to atheromatous change. Such calcareous deposits have been the subject of discussion ever since arterial disease has been recognized, and later it was shown that these processes remained not alone in the deeper layers of the intima, but advanced into the media.

Jores [5] and Matusewicz [6] have described another form of calcification in arteries, in which isolated patches of the intimal elastic lamina become the site of the deposit. I have met with similar examples, occurring most frequently in the iliac arteries.

Of late Moenkeberg [7] has described a form of calcification that he found in the media of arteries of the muscular type, and noted chiefly in the vessels of the extremities in old people on which clinicians base the diagnosis of arteriosclerosis. His findings are interesting, in that the arterial changes are often confined to these vessels alone, the aorta and its main branches being unaffected. He has found, however, that such degenerative changes do take place in the vessels of the extremities, when more or less arteriosclerosis (intimal disease) is present elsewhere in the body. His statistics show that the femoral artery is most frequently attacked, and that the process having started in one vessel spreads from the primary focus into the branches of the artery. Such a condition as a primary calcification of the media he did not note in any but the vessels of the extremities.

During the routine examination of vessels for degenerative processes in the arterial wall, with the newer technique now at our disposal, I was struck with the frequency with which microscopic calcium deposits were found when the macroscopic examination for the same was entirely negative. I very soon came

[5] *Ziegler's Beiträge*, 1897, xxi, 211.
[6] *Idem.*, 1902, xxxi, 317.
[7] *Virchow's Archiv*, 1903, clxxi, 141.

to the conclusion that the detection of pathological quantities of calcium by the naked eye was not to be relied upon. V. Kossa's method of staining the finer particles of calcium, particularly calcium phosphate, by means of silver nitrate, frequently gave a microscopic picture of calcium salts quite out of keeping with the opinion one formed from the macroscopic examination of the vessel. I have seen specimens in which the aorta was pliable and elastic, with not a trace of calcium salts to the naked eye, while the microscopic section showed the media to be loaded with calcium salts.

Leaving aside for the present the extent and nature of the calcium deposits in the intima, which secondarily invade the media, I wish to discuss the form of calcification found primarily in the media of the aorta and its larger branches, in other words in the vessels of the elastic tissue as distinguished from the muscular type. This form of calcification I have not met with in any other vessels; it differs thus from Moenkeberg's form, which occurs only in arteries of the muscular type.

The age of the patients in which primary calcification of the media of the aorta was found was usually over 65 years; I have encountered three cases under 45 years (aged 39, 41, and 43 respectively). So frequently does a medial deposit of calcium salts occur in old people that it might be ventured that all people over 65 years have it, and that in fully one third of those over 50 years is it present. Sex seems to make no difference.

In the majority of the cases the aorta is the vessel which is attacked, and from here the degenerative process advances into the carotids, innominate, and subclavian arteries. In the latter vessels the condition is as a rule less marked than in the aorta, where the calcareous salts are found uniformly distributed in the media in the different parts of the vessel. No site of predilection was noted in the aorta, but it was found that when one portion of the vessel was affected, calcium salts were found in the media and in other parts also.

It is the common finding that persons over fifty years of age show more or less arteriosclerosis, and hence it would be difficult to draw inferences as to the association of arteriosclerosis at that

time of life with the condition here described. In cases, however occurring before fifty years of age, there was a marked absence of gross arterial disease, so that this form of calcification of the media in the vessels of the elastic tissue type stands apart from what is ordinarily understood as arteriosclerosis. Nor is arteriosclerosis to be found in other parts of the body, though I have noted in some, not all the cases, the presence of calcified areas in the media of the vessels of the extremities as described by Moenkeberg.

Hence it would seem that the condition is a form of senile degeneration of the arterial system in which the media of the aorta and its larger branches are involved. This is further borne out by the microscopic examinations.

Macroscopically, as was stated, the vessel may show no change whatever, and the autopsy protocol invariably contains negative reports, except where a concurrent arteriosclerosis affects the other coats of the vessel. The aorta is elastic, not thickened, and the intima is smooth and glistening, while its surface shows a fine sprinkling of fatty granulations so common in advanced life. These slight intimal changes may be considered as the rule in old age, and although they do not represent a physiological process, they play no leading part pathologically. The naked eye inspection of the media and adventitia discloses no change in their structure.

The microscopic examination of frozen sections shows, particularly in the media, interesting changes.

The media is of normal diameter, and at first sight, in hæmatoxylin stained preparations, one is led to believe that no changes have taken place. A closer examination shows in the central zone of the media numerous clusters of fine dark-stained granules, which lie between the elastic fibres. Thick sections, at times, give the impression that the elastic fibres also contain these granules, an impression seen to be erroneous when thinner sections are examined: they lie only in the degenerated muscle fibres. It is to be noted, too, that the muscle tissue in the areas of the dark-stained granulation contains no nuclei, a point which is likely to be overlooked in a careless examination, as the granulation takes the place of the nuclei. In other such areas, which may be looked on as an early stage of the degeneration, the muscle cells are seen to have imperfect outlines, while the nuclei show stages of fragmentation. Amongst those degenerated muscle cells are to be seen, here and there, other cells which have retained their vitality, and hence give the impression that the granular deposit exists in them.

This is not so; more careful study shows that in no case are living cells to be found having within them this deposit of calcium salts. Nor have I noted in any of my specimens a calcification of the elastic fibres such as is found in the calcified media in the vessels of the extremities.

In one case I noted the presence of new formed blood-vessels in the media. While I take it as normal to have blood-vessels in the outer third of the media, yet the presence of numerous capillaries in the media, even though not accompanied by inflammatory infiltration, must be looked on as a reaction to some irritant. The presence of the blood-vessels in the media diminishes the quantity of calcium salts laid down in its vicinity, and the tissue lying about the vessels is entirely devoid of calcareous salts. And, further, the muscle cells lying near the new formed blood-vessel are not degenerated as are those in other parts. It would seem that with the presence of sufficient nutrition the deposit of calcium salts does not take place. This is further borne out by the absence of any deposit in the media bordering the adventitia, and that lying next to the intima. Thus the deposit of calcium is limited to the middle zone of the media.

The inner zone of the media, I take it, is nourished in part at least from the lumen of the vessel, through the intima. Koester[8] has shown that lymphatic spaces pass from the adventitia to and into the intima, and that inflammatory conditions advancing into the vessel wall pass from the adventitia towards the lumen. Confirming this view that some nutriment passes from within outwards into the walls of the arteries, I have noted that in thrombosed vessels, after a week, the intima contains no living cells of its former tissue, and that this tissue and the inner layers of the media are in a state of fatty degeneration. That is if the blood supply within the vessel is cut off, and an effective state of the vasa vasorum persists, the inner layers of the vessel, including the intima and inner strata of the media, become degenerated, and are only later replaced by connective tissue coming in with the newly formed blood-vessels from the adventitia.

Believing, then, that the degeneration and the later deposit of calcium salts in the media rest in part on the nutrition of that coat, it is seen that the greatest deposit of calcium takes place in the middle zone of the media. In the outer one third, where the media is well supplied with capillaries, and in the inner portions of the media supplied through the intima there is a marked absence of the degenerative process.

[8] *Berlin klin. Wochensch.*, 1876, p. 454.

Moenkeberg found in his cases of calcification of the arteries of the extremities, that the muscle fibres first showed a fatty degeneration. A similar fatty degeneration is also to be seen in the aortas of the cases under description. The fat granules are first found lying about the nuclei of the muscle fibres, while after the degeneration has advanced, and the cell has become filled with the small fat droplets, the nucleus becomes lost. The cell itself is later broken up, leaving the fat granules lying in the interstices of the remaining muscle cells. Thus it is often seen that the intact muscle fibres are surrounded by aggregations of these fine fat particles, which have been described as arising out of the lymph surrounding the cells. With the loss of more or less of the muscle tissue, by this process of fatty degeneration, I find a definite shrinkage at the site where they previously existed; on account of the blood pressure within the vessel, the laminæ are pressed closer together, and the remains of the fatty degenerated muscle fibres are found as clusters of fat droplets in the clefts between the remaining muscle cells. It is further to be noted that these aggregations of fat granules of former muscle cells are found lying next to the elastic fibres of the media, the elastic fibres themselves being unaffected. In none of the specimens that I have noted, in which a calcification of the media of the vessels of the elastic tissue type alone was present, have I seen fatty degeneration of the elastic fibres in the media. Thus it is seen that the disease affects primarily the muscle fibres.

With the modification of Fischer's method of detecting fatty acids and soaps (page 324) I was able, after decalcification of the specimens, to demonstrate that these same areas in the media are areas of advanced fatty degeneration. They give the reactions both for fatty acids and soaps.

That the deposit which I have demonstrated, by the use of silver nitrate, is calcium, is shown by its staining a dark blue with hæmatoxylin, its solution in hydrochloric acid, and the formation of crystals with sulphuric acid.

The best microscopic specimens demonstrating the calcium granules are obtained by the silver nitrate method, in which

the granules are seen to be arranged in clusters parallel to and often closely bordering upon the elastic fibres. The elastic fibres are always to be traced as uninterrupted strands through these areas of degeneration. That the elastic fibres resist degenerative processes to a greater extent than the muscle fibres, I have noted also in other arterial diseases. Thus, too, in the calcified areas of the arteries of the extremities the muscle fibres are the first to be affected, and in the early stage the calcification of these fibres is similar to the process in the muscle fibres in the aorta. The muscle fibres undergo an early fatty degeneration, which is later followed by a deposit of lime salts. As the calcification of the muscle cells advances the granules of lime salts become confluent and form larger masses. In the calcified arteries of the extremities, described by Moenkeberg, it is seen that the outer border of the calcareous deposit has a similar appearance to the calcification in the arteries of the elastic tissue type, the difference between them being that in the former the process goes on much further, attacking the elastic fibres and later forming macroscopic deposits of calcium. Macroscopic deposits of calcium in the media alone of the aorta I have not so far encountered and am led to doubt if they ever occur.

The process of calcification in itself is similar to that found in other degenerative processes. One has to do essentially with a fatty degeneration of the specific cells, followed by death of the cell and a fatty acid stage, in which calcium soaps are laid down. A later conversion of the calcium soaps into the calcium phosphate and carbonate then results.

The intima, as I have said, showed no macroscopic change except in such cases as exhibited a concurrent intimal arteriosclerosis, and in these latter cases the medial disease was equally pronounced in the portions of the aorta lying beyond the intimal plaques. However, in all cases showing calcareous deposit in the media, the intima was found to be fatty, the degeneration being most marked in the deep, or musculo-elastic layer. In the early cases the changes in the intima are mostly a hypertrophy of the musculo-elastic layer, with more or less of a fatty degeneration attending it. The connective tissue layer of

the intima is not thickened, neither does it show any degenerative changes. Similarly no constant characteristics were noted regarding the elastic fibres in the intima, which at times showed an increase in the lamellæ of the internal elastic lamina, while at other times this lamina showed fatty changes. The changes in the elastic fibres in the intima were related more or less to the character and condition of the elastic fibres in the media, being increased when the latter were in a state of granular degeneration.

In conclusion I would say that the calcification of the media of vessels of the elastic tissue type resembles that found in the arteries of the extremities (the vessels of the muscular type) in that the process of calcification is confined to the media, but differs from it in that the deposit of calcium salts does not go beyond the granular stage, and the muscle fibres alone are involved in the process of degeneration, the elastic fibres remaining unaffected. Macroscopically there is no change in the media, while microscopic sections show the media to be loaded with a granular calcium deposit. This process, which stands in close relation with the nutrition of the vessel wall, is a common one in persons over fifty years of both sexes, and almost constant in the aortas of those over sixty-five years of age.

STUDIES UPON CALCAREOUS DEGENERATION.

By OSKAR KLOTZ.

STUDIES UPON CALCAREOUS DEGENERATION.*

V.—THE RELATION OF EXPERIMENTAL ARTERIAL DISEASE IN ANIMALS TO ARTERIOSCLEROSIS IN MAN.

By OSKAR KLOTZ,
Resident Pathologist, Royal Victoria Hospital, Montreal.

PLATE XXIV.

Since Josué first produced arterial changes in rabbits by means of adrenalin there has been a whole host of experimenters who have repeated and confirmed his work. Although Josué was the first to produce arterial lesions by means of adrenalin, he was preceded by Gilbert and Lion who were successful in bringing about arterial changes, by means of bacteria and their toxins; these lesions they believed simulated arteriosclerosis in man. Sumikawa, too, caused inflammatory changes near the arteries in rabbits, and believed that the vascular changes were identical with those found in arteriosclerosis. However, there is no doubt that arterial changes have been produced with most success by the inoculation of adrenalin. Erb, Baylad and Albarède, Külbs, Fischer, Scheidemandel, and Lissauer have experimented with adrenalin, and have noted the vascular changes. In the main their observations agree, but details of minor importance have been emphasized by some of them. B. Fischer has further found that digitalin when inoculated intravenously produces lesions similar to those caused by adrenalin.

The French workers, Josué and Baylad and Albarède, are most emphatic in maintaining that the intimal changes found in the vessels of experimental animals are of prime importance. The others, with whom I agree, hold that the medial lesions are the principal changes caused by adrenalin. The intimal changes,

* This study was carried out at McGill University, Montreal, under a grant from the Rockefeller Institute for Medical Research.

which are rarely found, are of a secondary nature, or else are brought about by a process quite distinct from that producing the medial changes.

On account of the importance of a proper interpretation of these experimental lesions, and of the relation which they bear to the diseases of the arteries in man, I feel justified in making a histological comparison of the experimental and human lesions. It is impossible to call the macroscopical changes in the rabbit's aorta atheroma or arteriosclerosis unless the histological examination supports this view. I do not doubt that some have been misled in the use of these terms by the naked-eye appearance of the intima, while others have used the term arteriosclerosis in its broadest sense. Should the latter course be adopted, it should be recognized that experimental arteriosclerosis in rabbits is entirely distinct from sclerosis of the aorta in man. Moreover, as I shall point out later, the term atheroma should not be used in connection with the adrenalin lesions.

It is unnecessary for me to give in detail the methods of experimenting on animals, further than to state that my methods resemble those of previous investigators and more particularly those described by B. Fischer. The pure solution of adrenalin chloride, one in a thousand, was injected into the ear veins of rabbits.

Microscopical lesions, such as dilatation of the blood-vessels, hæmorrhages in the brain, and aneurismal distentions of the larger vessels, were noted. The most frequent lesions were situated in the descending arch of the aorta, where white calcified plaques formed the chief naked-eye alterations. The aneurisms were found to be situated in the centres of these white plaques which have been described by some as atheromatous areas. Histological examination shows that these plaques, at an early stage, are situated entirely in the media and that the calcification which occurs here is the secondary result of a degenerative, chiefly fatty, change which takes place in the media. Calcification is never found in the intima, and only rarely are there any intimal changes. The intimal changes which occur are of two kinds: either there is a

local heaping up of endothelial cells at a point where no degenerative changes of any kind have taken place, or there is thickening of the intima underneath the endothelial cells, chiefly due to proliferation of the connective tissue, in which slight fatty changes may occur. Such being the case, the term atheroma must be discarded in describing the experimental arterial lesions in rabbits.

As I have pointed out elsewhere, the primary change in the media, preceding the stage of calcification, is fatty degeneration of the muscle cells, followed by fatty degeneration of the elastic fibres. In the earliest lesions, before any macroscopic change can be noted in the vessels, fine granules of fat are aggregated about the nuclei of the muscle fibres. These granules of fat have an arrangement not unlike that seen in fatty degeneration of the heart muscle. From this stage of degeneration the process advances rapidly so that in a few days the median zone of the middle tunic of the vessels is in an advanced stage of degeneration. Fischer has already noted the rapidity with which the muscle fibres change under the influence of adrenalin inoculation, and hence has applied the name arterionecrosis. Since the degenerative changes are not so rapid as to prevent the appearance of fatty degeneration, and since there proceeds with the apparent necrosis a process of calcification, the name proposed by Fischer is a little misleading. I must grant that sections stained with hæmatoxylin and eosin give a picture of an active necrosis, but with Weigert's stain alone, or combined with Sudan III, the necrotic appearance disappears.

My studies lead me to accept the histological descriptions given by Fischer and others, except, as I have stated, that I find fatty changes in the muscle and elastic fibres preceding the deposit of the calcium salts. The mode in which these calcium salts are laid down has also been discussed in full in another paper, but as regards the sites of the calcium deposit I wish to speak more fully.

In sections stained with hæmatoxylin and eosin, it is seen that the lesion lies in the middle zone of the media and that in the early stages the muscle fibres become granular and lose their nuclei. With this degeneration of the muscle fibres, which must

necessarily be accompanied by softening of the tissue, the elastic fibres are packed more closely together, leaving less room between them for the muscle cells. At these points, where there is a loss of the muscle elements, the vessel wall is distinctly weakened, and partially gives way, leading to small aneurismal sacculations. In advanced lesions these aneurismal dilatations are the most marked macroscopic changes that are found.

When the muscle cells in the media have undergone fatty degeneration, the condition advances, until the cells become fairly loaded with the fat granules. Nuclear degenerations then become apparent and soon the cells die, leaving only their outline marked by the deposit of the fat granules which were in them. These fat granules do not coalesce but remain as isolated particles. Passing through the stage of fatty acids and soaps[1] they are converted into calcium compounds of the fatty acids, and hence the calcium deposit occurs as a fine sandlike material distributed in the sites of the former fatty granules. Following closely upon this change, the elastic fibres also become fatty, as Jores has observed, and go through the same process of calcification as the muscle cells. There is now a fine granular deposit of calcium at the site of the muscle fibres and rigid zig-zag calcified elastic fibres lying between them. This picture is not seen in the advanced stages of the calcification of aorta in rabbits, for the calcium salts have coalesced more or less to form larger masses, so that the former histological structure of the media is entirely lost. In no instance of experimentally calcified vessels in animals has primary calcification of the intima been noted. Occasionally I have seen very advanced experimental arterial disease in which the calcification of the media extended immediately below the intima.

If we are to accept Jores's definition of arteriosclerosis, namely, that the disease consists of a hyperplasia with degeneration of the musculo-elastic layer of the intima as seen in the human aorta, these experimental lesions would not fall in this category. I am not, however, inclined to accept this restricted

[1] See the studies upon calcareous degeneration by the writer. *Jour. of Exper. Med.*, 1905, vii, 633.

definition of arteriosclerosis, particularly as regards the human disease.

Lissauer compares the experimental lesions in arteries of rabbits to syphilitic arterial disease in man, pointing out that syphilitic arteritis attacks particularly the media. He falls short of a proper comparison. The main factor in the experimental lesions is the calcification of the muscle and elastic fibres in the media, and this never takes place in syphilitic disease of the vessels. In syphilitic arteritis the main reaction, consisting of an inflammatory change with new formed blood-vessels, an infiltration of small cells, and later the production of connective tissue, takes place in the outer third of the media. The blood vessels affected by adrenalin, on the contrary, show no reparative reaction in the media, the process being entirely a degenerative one. If a reaction does occur, it is secondary to a medial degeneration, and this reaction consists of a heaping up of the endothelial cells in the intima.

I would point out that although the lesion does lie in the media—and especially in the middle zone of the media—there are no changes in the vasa vasorum. All the hypotheses, maintaining that the action of adrenalin on the vasa in the vessel wall is the prime factor in the production of the necrosis, are based upon general inferences regarding the action of this drug in other parts; the histological appearances give no support to this theory.

A second arterial disease which Lissauer wishes to compare with the vascular changes caused by adrenalin is the so-called neurotic angiosclerosis. He points out that Lewaschew and also Fraenkel found that by injuring or cutting the nerve supply of vessels, they became infiltrated with connective tissue, and that the change lay only in the media. Their results are quite contrary to those of Jores, who found that neither injury to a nerve nor the complete severance of it had any effect on the histological structure of the vessel it supplied. In syphilitic arterial disease, as I have mentioned, and in the neurotic lesion, calcification of the media is never seen.

There is, however, an arterial disease in man which simulates the

experimental arterial degenerations in every respect. This disease I have spoken of elsewhere as Moenkeberg's type of arteriosclerosis. This disease, as Moenkeberg and others have pointed out, affects the vessels of the extremities—the vessels of the muscular type—that is, arteries in which the muscle tissue predominates in the media. From this lesion the clinician makes the diagnosis of arteriosclerosis when he finds the radials hardened like "pipe stems" or "beaded like a trachea." It is now definitely known that this form of arterial disease is distinguishable from arteriosclerosis as we find it in the human aorta. Moenkeberg's type of arteriosclerosis affects the media alone, causing degenerative changes. The intima is affected neither by degenerative nor by reparative changes, or if such changes are present they are secondary to or parts of another disease and not associated with changes taking place in the media. This disease of the media affects the vessels of the extremities—the femorals, tibials, internal and external iliacs, radials, and brachials being particularly susceptible.

Preceding the deposition of calcium salts in the media, there are degenerative changes which consist chiefly of fatty degeneration, which is visible to the naked eye, and can be distinctly traced in the media when the vessel is cut open longitudinally. This early fatty degeneration in the media differs from that of arteriosclerosis of the aorta in that the fatty plaques do not stand above the surface of the intima, but can be seen through this membrane. One is convinced by the microscopical picture, that in the early stages the muscle cells undergo a fatty degeneration characterized by the deposition of the finest fat globules. The calcium salts, too, are found to be deposited as fine sandlike granules in the degenerated muscle fibres. In the early stages of degeneration, calcium granules are found in double wedge-shaped aggregations lying between the elastic fibres, and this grade of calcification is not to be recognized by the naked eye. However, with the constant accumulations of fresh calcium deposit, the granules lie very close together and then coalesce. At this stage, when the calcium granules are becoming densely aggregated, we find that the vessel wall decreases in thickness; there is a thinning of

the media with a bulging outward of the wall at this point. Distinct multiple aneurismal dilatations are of common occurrence in these vessels, so that the outer contour of the vessel is quite irregular. From the inner surface of the vessel it is seen that the calcified areas form depressions which are lined by smooth and unchanged intima. Microscopically, the elastic fibres are seen to be stretched into straight lamellæ and no longer show the wavy character found in healthy vessels. The elastic fibres, too, are calcified and are lying more closely together, although in advanced cases this is difficult to distinguish, as the calcium deposit in the muscle and elastic fibres have run together into a solid mass. In consequence of the constant tension and stretching of the elastic fibres, they have become calcified in the position that they had in these vessels, and thus we find the straight calcified laminæ. Where calcification of elastic fibres has advanced beyond the general area of calcareous degeneration, and, too, where contractile muscle fibres have persisted between them, these elastic fibres have taken on a zig-zag outline due to fractures.

Microscopically one can demonstrate that the running together of the calcium granules takes place in the middle of the media, and that at either end of such a mass are outrunners of calcified elastic fibres and granular degenerated muscle fibres. The internal elastic lamina is stretched as it passes over the calcified and aneurismal portion of the media.

The intima occasionally shows some reaction over the areas of medial degeneration, the endothelial cells being heaped up. This intimal change is, however, entirely secondary and has nothing to do with the primary cause of the medial change, and further is not a necessary accompaniment of the medial degeneration.

Moenkeberg holds that the adventitial changes are in great part confined to thickening of the intima of the vasa vasorum, but points out that this limitation is not a constant feature in the disease. There are some who claim that the degeneration of the media is due to occlusion of the vasa vasorum causing poor nutrition of the media. However, the changes in the vasa vasorum are too inconstant to allow us to draw any definite con-

clusion, and, moreover, it is very hard to estimate the amount of change in these small arterioles with no definite wall, especially when they are collapsed and empty.

Pure medial calcification is commonly found as an accompaniment of advanced age, occurring most frequently after the age of fifty. But, as Marchand has pointed out, it occurs also in young individuals. The disease affects males more frequently than females, the ratio being about six to one. It is further of interest that medial calcification occurs in the vessels of those extremities which are most active; thus in right-handed individuals it is found more pronounced on the right side, while in those whose duties keep them constantly on their feet the femorals are most affected. Again, the disease is particularly common among the laboring classes.

When the experimental lesion in animals and the medial arteriosclerosis in man are compared, they present great similarity in their anatomical, histological, and, it may be, their etiological characters. In each case the essential lesions are confined to the media, the changes in the intima being of a secondary nature or else the result of a different disease. In both lesions the process in the media rapidly leads to calcification of the muscular and elastic elements, fatty change preceding the deposition of the calcium salts. Aneurismal dilatations at the sites of the calcium deposits in the media often occur with both lesions, and in the dilated part the vessel wall is much thinned as the result of the loss of muscular elements and the packing together of the elastic fibres.

Both diseases are brought about or accompanied by an increased blood pressure; in the one it is artificially produced by the use of adrenalin and involves the general circulation, while in the other it is localized in parts, the extremities, where increased work, straining and constriction of the muscles, lead to heightened pressure. Whether this heightened blood pressure acts directly on the vessel walls to cause the medial degeneration, or whether by the stretching of the arteries the vasa vasorum are in part or wholly pinched off, leading to focal necrosis in the media, is not wholly clear. Certainly from the focal character

of the experimental lesions and from the annular arrangement of the degeneration in the vessels of the extremities in man, I should feel very much inclined to think that the vasa played an important rôle in the production of these changes. However, we must not lose sight of the fact that in the experimental animals the toxic character of the substances inoculated may be an important factor in the production of the lesions, as Fischer has pointed out.

Both of these lesions must be distinguished from the intimal form of arteriosclerosis as found in the aorta, and the presence of one condition does not indicate the presence of the other.

Several clinicians have made the observation that when pure medial arteriosclerosis is present in the vessels of the extremities without any changes in the aorta there is no hypertrophy of the left heart, while, on the contrary, if the aorta is affected with arteriosclerotic changes the heart shows hypertrophy. This fact suggests that while the aorta is healthy and elastic, it relieves, to a great extent, the increased blood pressure which would naturally follow the hardening of the peripheral vessels, while, on the other hand, if the elasticity of the aorta is destroyed, the increased pressure is directly transferred to the heart, leading to hypertrophy of the organ. The experimental hardening of the walls of the aorta apparently confirms this view, since hypertrophy of the heart frequently occurs with it.

BIBLIOGRAPHY.

Baylad and Albarède.—*Comp. rend. Soc. de Biol.*, 1904, lvii, 640.
Erb.—*Archiv für exper. Path.*, 1905, liii, 173.
Frænkel.—*Wiener klin. Woch.*, 1896, ix, 147.
B. Fischer.—*Zeitsch. für Psychiatrie.*, 1904, lxii, 241.
B. Fischer.—*Verhand. d. Kongress f. innere Medizin*, Wiesbaden, 1905, xxii, 235.
B. Fischer.—*Deutsche med. Woch.*, 1905, xxxi, 1713.
Gilbert and Lion.—*Compt. rend. Soc. de Biol.*, 1889, xli, 583.
Gilbert and Lion.—*Archiv. de med. exper.*, 1904, xvi, 73.
Josué.—*Presse médicale*, 1903, xi, 798.
Klotz.—*Journal of Exper. Med.*, 1905, vii, 633.
Külbs.—*Archiv. f. exper. Path.*, 1905, liii, 140.
Lissauer.—*Berlin. klin. Woch.*, 1905, xlii, 675.
Lewaschew.—*Virchow's Archiv*, 1903, xcii, 152.
Marchand.—*Eulenberg's Realencyclopædie*, 1894, Bd. ii.
Moenkeberg.—*Virchow's Archiv*, 1903, clxxi, 141.

Fig. 1.

Fig. 2.

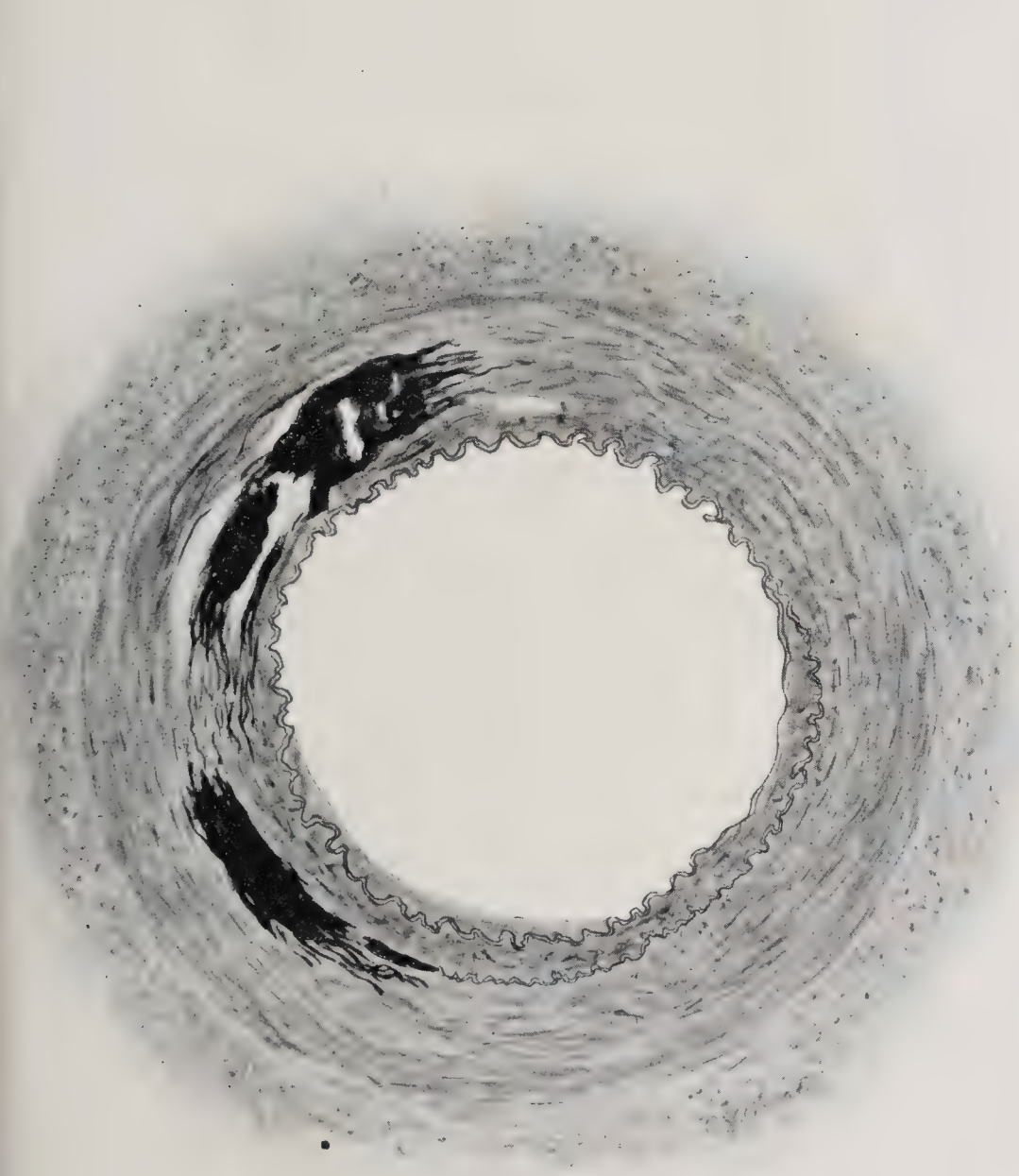


Fig. 3.

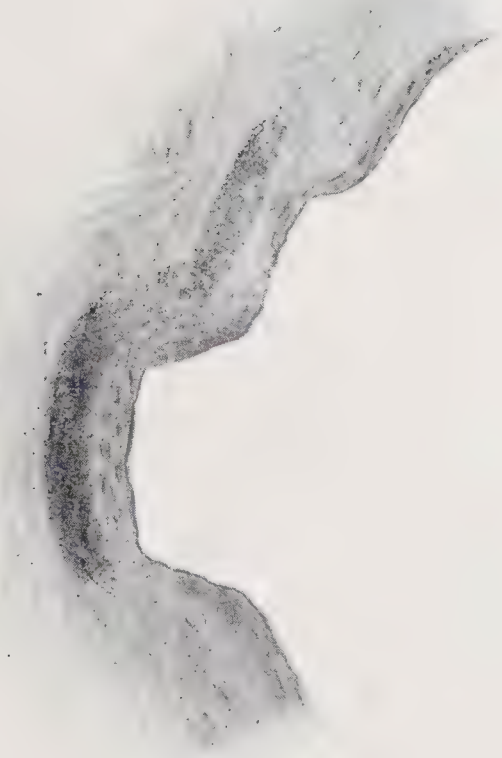


Fig. 4.

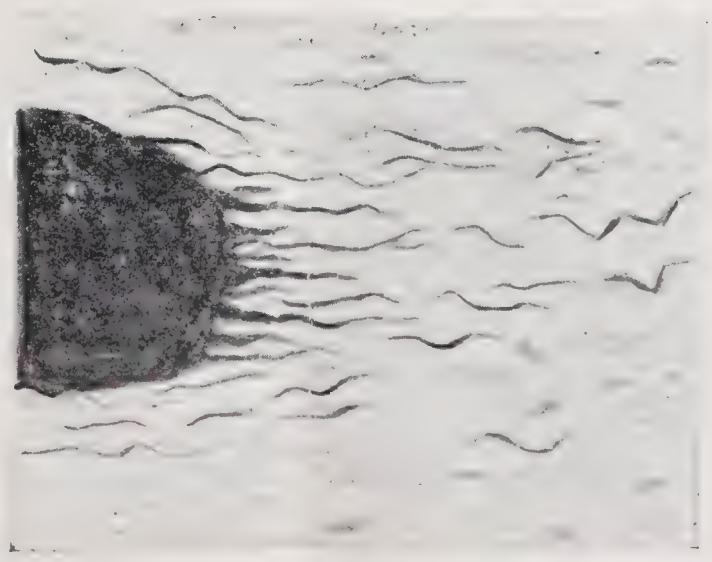


Fig. 5.

Pearce and Stanton.—*Trans. Assoc. of Amer. Phys.*, 1905, xx, 513.
Scheidemandel.—*Virchow's Archiv*, 1905, clxxxi, 386.
Sturli.—*La semaine médicale*, 1905, xxv, 128.
Sumikawa.—*Ziegler's Beiträge*, 1903, xxxiii, 242.

EXPLANATION OF PLATE XXIV.

Fig. 1.—Femoral artery (natural size), showing pittings and small aneurismal dilatations in the areas of calcification of the media.

Fig. 2.—Aorta of rabbit (enlarged x 2), after injections of adrenalin, showing small calcified plaques with depressions and aneurismal bulgings in their centres.

Fig. 3.—Femoral artery (low power, Leitz obj. 3, ocular No. 2), showing calcification of the media with little change in the intima other than splitting of the internal elastic lamina.

Fig. 4.—Aorta of rabbit after injections of adrenalin (low power), showing calcification in the media with no change in the intima. Vessel wall shows aneurismal dilatation.

Fig. 5.—Femoral artery (high power), showing densely calcified media, along with a less intensely calcified area in which the elastic and muscle fibres are in the early stages of calcification.

OSTEOMALACIA ASSOCIATED WITH LIPAEMIA.

BY

OSKAR KLOTZ,

Resident Pathologist, Royal Victoria Hospital, Montreal.

Reprinted from the Montreal Medical Journal, August, 1906.

OSTEOMALACIA ASSOCIATED WITH LIPÆMIA.

BY

OSKAR KLOTZ,

Resident Pathologist, Royal Victoria Hospital, Montreal.

The following two cases though incomplete in their study are of such interest as to merit a report of them. It is regrettable that as the condition of lipæmia was not recognized during life, we are unable to link the *intra vitam* findings with those obtained at post mortem; and, moreover, that the qualitative blood examinations are wanting in each case.

At the present time the question of fat absorption and fat destruction is occupying a prominent place among the subjects of research, and recent findings go more and more to prove that the transformation and transportation of fat in the various parts of the body follows common chemical laws; that is, that the process of absorption from the intestinal canal into the blood stream differs but little from the fat absorption at the natural fat depots of the body; each being the result of enzyme action. And so too it has been shown that fat emboli, so called, which are so common in the lungs after injury, are of little consequence there as they become absorbed by the lipases of the blood thus again freeing the blood vessels. However, it is still a debatable question whether the kidneys are able to excrete fat, and the manner in which fat is secreted by the mammary glands is also undetermined.

Cases of lipæmia have not been uncommon in association with diabetes, and diseases of the pancreas; in neither of our cases, however, was there true diabetes present, though the first one showed the presence of small quantities of sugar in the urine just before death.

History of Case I.— J, female, aged 24. From the service of Drs. Garrow and Archibald. The patient had been in hospital previously for osteomalacia and genu varum, and during her stay developed erysipelas. She was discharged on April 8th, 1905. After returning home she remained fairly well up to May 12th, when she developed a severe pain in the right side; this pain continued to increase in intensity. Previous to onset of pain she had some diarr-

hœa, which was not very severe. On May 15th she had some difficulty of micturition. She vomited only once. On admission patient was suffering very great pain, referred to the right side. Examination of the abdomen showed no tenderness or rigidity on left side, but these signs were present on the right. There was some fulness of the abdomen which extended from the umbilicus towards the antero-superior spine, and in this region a large and somewhat round mass could be palpated. An operation was undertaken and the abdomen opened over the appendix. A large globular cyst-like mass was exposed, and free pus escaped from it. Numerous adhesions were encountered in the pelvis, and the mass was made out to be a pus cavity in the broad ligament. The uterus was noted to be of infantile character. Total ablation of the broad ligament on the right side was performed. Patient stood the operation fairly well, but was very weak. For four days the patient's condition remained about the same. On May 20th (five days after operation) vomiting became fæcal in character. There was heavy albumen in the urine and hyaline casts. For the next three days the fæcal vomiting continued, and the abdominal wound showed absolutely no attempt at repair. There was no evidence of peritonitis. May 26th. Patient had been gradually sinking, and appeared irrational at times. Towards the evning she became comatose, but regained consciousness shortly afterwards. While conscious she was quite noisy. At midnight patient was seized with a severe convulsion resembling that of a uræmic condition. Between the convulsive attacks she regained consciousness. During the night she had some twelve convulsions, and died at 10.10 the following morning.

Autopsy performed by Drs. Adami and McCrae.

At autopsy was found the body of a rather dwarfed girl with a small barrel-shaped chest. The left leg had a marked bending of the femur with the convexity outwards. There was a posterior bending of both tibiæ, and left lateral scoliosis in the lumbar region. It was noted on removing the skull, as also in cutting the other bones that the bone cut easily, and could be pared with a knife. The pelvis was a marked example of osteomalacia; the armi of the pubes joined at a very acute angle, so that 5 cm. from the symphysis they were only 2.75 cm. apart; the transverse measurement of the brim was 8.5 cm. The long bones were found to be composed of only a shell of bone on the outside with a honeycombed structure immediately within this, and containing a soft pulpy marrow. The ribs have a very marked amount of spongy tissue surrounded by a thin shell of bone while they and the spinal vertebræ can be cut with moderate ease with

a knife. Of the general organs in the body the kidney showed a condition of chronic parenchymatous nephritis while a local septic peritonitis was also present.

Anatomical diagnosis.— Right salpingo-oophorectomy: acute localised septic peritonitis; chronic parenchymatous nephritis; osteomalacia; uræmic ulceration of œsophagus and jejunum; acute catarrhal gastro-enteritis; pulmonary apoplexy; lipæmia, uremia (?).

In the microscopical examination the following conditions were noted:

Lung.— The vessels of the lung were found to be loaded with fat staining material. However, it was not universally seen that the alveoli are bounded by capillaries loaded with fat. The walls of the blood vessels, too, were noted to contain fat in minute granules, which lie both in the endothelial and connective tissue cells of the vessel walls.

Liver (Sudan III and Hæmatoxylin).— Showed an extremely interesting condition in that the lobules are picked out by the Sudan in the peripheries, the central vein and its neighbourhood being entirely free from fat. This fat was present within the liver cells themselves, there being little or none found free in the capillaries. The hepatic artery showed little fat within it. Many of the bile ducts were seen with their cells containing fat granules both at their periphery and towards the protoplasm adjoining the lumina. The larger portal vessels too contain some fat. The liver cells are seen to contain fat in minute granules which do not seem to coalesce readily.

Heart muscle.— There is fatty degeneration of the individual muscle fibres with a considerable quantity of fat in the small capillaries and present in and about the cells of the larger blood vessels.

Kidney.— Fat is found in the capillaries of the Malpighian tufts and besides this in the convoluted tubules. Many of the epithelial cells of the convoluted tubules were seen to be desquamated, having their substance densely infiltrated with fat. The straight tubules too showed a fatty change, in that fat droplets were seen in the base of the cells.

Spleen.— The vessels showed a considerable quantity of fat within them, and the cells also contained the fine granules of fat.

Case II.— F. B., æt. 24. From the service of Dr. W. F. Hamilton. Patient was in usual health on May 14, 1906, and went to bed feeling quite well. At midnight he was found by the people in the house unconscious and rigid. He had evidently fallen out of bed. He soon regained consciousness and complained of soreness in the muscles of his arms. Between midnight and 10 a.m. the following morning he had three convulsive seizures, regaining consciousness in the intervals. Since 11 a.m. he has remained unconscious.

Patient was born in England and had been in Canada about a year. He has always been a delicate child and suffered from frequent attacks of nausea and vomiting. He has had indefinite pains in the legs which would become stiff, causing a spastic gait. He had difficulty in raising himself after stooping. This stiffness and pain of the legs extended into the arms. He is an excessive smoker but does not use alcohol. On admission to hospital his breathing was quiet and regular. Thorax showed nothing unusual. Hæmoglobin amounted to 55 per cent.; leucocytes, 20,000. There is a marked genu valgum recurvatum of the right knee. Patient became comatose and could not be roused. On 16th May, 1906, he had ten convulsive seizures up to 4.30 a.m., when they began to increase in depth; his right arm became rigid and wrists flexed. There was no frothing of the mouth, nor biting of the tongue. There had been Cheyne-Stokes breathing since 7 a.m. Patient died at 11 a.m. Some albumen and casts were found in the urine, but no sugar or blood.

An autopsy was performed by Drs. McCrae and Klotz.

At post-mortem was found the body of a young man of medium stature in whom the shoulders were apparently large, elevated looking, not unlike an upward dislocation of the humeri. The legs were bowed backwards and showed the condition of genu valgum recurvatum The skull cap was rather thin and cut readily, and when removed the calvarium seemed to be mostly made up of diplöe. The right humerus had the greater tuberosity fractured, and a shell of bone about the size of a 50 cent piece lay displaced on it. The surgical neck of the bone, too, was broken with some displacement upward of the upper fragment. The medulla of the shaft of the humerus was very pulpy, and was converted almost entirely into red marrow, there being only faint streakings of yellow marrow remaining in it. The left humeri was completely broken through at the surgical neck where the compact bone existed only as a thin shell. With a little force the shaft could be fractured in new places. At the sites of the original fracture of the humeri there was found blood infiltration of the muscle and surrounding loose tissue, denoting the recent occurrence of the lesions. The vertebræ were soft and could everywhere be cut with a knife. The bent tibiæ showed nothing further beyond the bending of the bone and the characters of the thinning of the compact tissue and transformation of the marrow as noted in the humeri.

Frozen sections of the different organs stained with hæmatoxylin and Sudan presented very interesting features.

In the lung a great amount of fat was found in the small capillaries bounding the alveoli and appearing as if these vessels were plugged.

The fat existed in one continuous mass, passing from the larger to the smaller vessels. A condition of isolated plugging of vessels by means of fat at their bifuracation was not seen. In some instances the fat could still be recognized in very minute and sand-like droplets. The kidney had the capillaries of the cortex and median zone filled with fat, the Malpighian tufts having their capillaries distinctly defined by the Sudan stain within them. The larger vessels of the kidney also showed fat in their lumina. The parenchymal cells of the cortex were in a state of fatty degeneration, particularly to be noted in the convoluted and collecting tubules.

Anatomical diagnosis.—Osteomalacia, chronic mixed nephritis, lipæmia, cloudy swelling of liver and kidneys, fracture of both humeri with hæmorrhage into muscles, genu valgum recurvatum, uremia (?).

The chief interest in the above two cases is the association of osteomalacia with lipæmia. The condition of osteomalacia extended through the entire osseous system—as far as could be examined. The vertebræ and skull in both cases were markedly affected, while in Case II the long bones were also severely involved in the disease, so that spontaneous fracture occurred in the humeri.

In each of the above cases the occurrence of the excess fat in the blood, is with difficulty coupled as a complication or process of the osteomalacia, as in each case there was present another condition, which, although small in itself, must not be overlooked. We had in the first case the presence of sugar in the urine, arising shortly before death. Small as was the amount of sugar which was present, the fact cannot be entirely outlawed, on account of the frequent occurrence of excess fat in the blood in diabetes.

In Case II we had the presence of spontaneous fracture of both humeri which might be held to account for the fat found in the capillaries of all the organs. However, the distribution of the fat in the organs does not support the contention that a large quantity of fat was suddenly thrown into the circulation of the occurrence of spontaneous fracture. Had this been the case we should have expected to find the greater quantity of fat lodged in the lungs, instead of being fairly uniformly present n all the vessels.

It has been shown experimentally that large quantities of fat thrown into the venous system lead to a blocking of the radicles of the pulmonary artery, with a consequent respiratory death. At the same time it is the frequent finding at the post mortem table to note the presence of fat in the vessels of the lung after all fractures and severe injuries of bone. Usually, however, when death follows such an in-

jury the lungs are the only seat of the fat deposit. In Case II where the patient died within a short time after the fracture of the humeri, we would not expect to find the large quantity and the wide distribution of the fat.

Similarly in Case I. I know of no reported case in which, with the small quantity of sugar present in the urine, and occurring only a few hours before death, there has been an excess of fat found in the blood. Nor are we justified in speaking of the fat deposits seen in the capillaries after death, as emboli, for in no organ is there the end result of an embolus,—an infarct. The wide distribution of the fat in the blood gives us clearly the picture of lipæmia.

Quite recently Turney and Dudgeon reported a case of diabetes in a female aged 35, in whom the disease had been existing eighteen months. Nothing unusual was noted in the course of the diabetes until the eyes were examined. The condition of the fundi which were alike was striking. The discs were pale, the vessels were filled with a milky fluid instead of blood. The patient did not complain of her sight. On examination of the blood it was found that there was a remarkable quantity of fat present in it. The interesting feature of this case is that although much of the blood was replaced by free fat, the condition did not produce any untoward symptoms. This agrees with the observations of Fischer, who reported a most extreme case of lipæmia in a diabetic patient. During life the blood appeared as a milky fluid, and Fischer found that the fat was present as very minute particles, smaller than red blood cells, so that no interference was encountered in the capillary circulation. Fischer also noted that it was only after the death of the patient that the fat particles ran together to form larger globules. It was these larger globules that gave the appearance of fat emboli in the vessels.

The condition of true lipæmia can be reproduced in animals without producing in them any such symptoms as are encountered in pulmonary embolism. The best emulsion of fat which we can use in the experiment is milk, which can be inoculated into the veins of rabbits until the circulating blood holds an appreciable quantity of fat, without producing any serious effects in the animal. As was said above, the inoculation of the pure fat, containing sufficient olein to make it liquid at body temperature, into the veins, produces quite different symptoms from those produced by milk. In the former where the fat already exists in larger and smaller globules, the vessels of the lungs become rapidly plugged, and, moreover, the cohesion of the fat to the walls of the blood vessels helps in sealing off the small arteries. In these

experiments it is found that little fat reaches the general circulation, almost the entire quantity being held in the lung capillaries. Should death not occur, after the inoculation of fat, the emboli are slowly dissolved by the lipase of the blood, besides being broken up into smaller globules by the endothelial and connective tissue cells growing into them. Wuttig found that these cells extended long processes into the fat masses and tended to reduce them into smaller particles.

The observation of Fischer, that the fat emulsion as is present in lipæmia, only tends to coalesce into larger fat masses after death, is an interesting one. It would seem that the fat diaplets are protected by a coating which keeps them from running together, and most likely the coating consists of a layer of fatty acids or soaps or their compounds with the proteid.

Under the general heading of osteomalacia a number of different diseases have been listed. Among these, Schönberger, Grawitz, Marchand and others have described a form in which the bone changes appeared secondary to a diseased condition (tumour) of the medulla of the long bones. In Schönberger's case, the tumour masses had a predilection for the bony structures and were distributed sporadically in the different bones. The histological examination of cases showed the tumour masses to consist of giant-celled sarcoma.

Such cases of tumour invasion in the bones, with secondary rarefaction of the osseous tissue are to be differentiated from the true osteomalacia which is primary in the bones. In the former cases the osteomalacia is limited to the region of the tumour growth, in the latter the entire skeleton is involved.

The true osteomalacia occurs most frequently in young women during or after pregnancy, and it is apt to begin in the bones of the pelvis, where it remains the most marked. Successive pregnancies in these cases aggravate the condition, so that the whole skeleton is converted into a non-calcified flexible,— in other cases fragile, tissue. The non-puerperal form is noted most frequently in the vertebræ and thorax, spreading then to the extremities and finally to the cranial bones.

The incidence of the disease is practically limited to certain geographical areas; in Germany, in particular, it is confined to the basin of the Rhine (Ziegler).

Eisenhart found that the alkalinity of the blood was reduced in osteomalacia, while v. Recklinghausen placed more stress on some vascular derangement of the bones. It has been held, too, by some that the loss of the lime salts in osteomalacia is consequent upon the formation of excessive lactic and carbonic acids in the bone tissue. There

appears to be something in common between osteomalacia and rickets, as in each the non-calcification of osteoid tissue is a prominent feature.

In the cases here reported, we have an example of each of the two forms of osteomalacia. In the first case the framework of the bones was abundant, but was lacking in calcium salts; in the second case there was no such deficiency of lime, but the trabecular framework was scanty. In each case, however, red marrow could be squeezed from the different long bones. and they had the common feature of being both associated with lipæmia.

Another interesting feature which, however, in both of our cases remains unexplained, is the occurrence of convulsions just before death. Whether these were the result of uræmic intoxication or some blood dyscrasias connected with the osteomalacia, cannot be said.

ON THE EXPERIMENTAL PRODUCTION OF ARTERIO-SCLEROSIS.

BY

OSKAR KLOTZ, M.D.

Lecturer in Pathology, McGill University; and Resident Pathologist, Royal Victoria Hospital, Montreal.

Before taking up the study of the experimental production of arterio-sclerosis it is necessary to ask, What is arterio-sclerosis? (*a*) Is it an entity; or (*b*) are several distinct morbid conditions included under this one heading; or (*c*), what comes nearly to the same thing, in different states which we are accustomed to regard as arterio-sclerosis, do we find the different coats and constituents of these coats affected diversely?

It is necessary, to ask these questions, because, as I shall show, different procedures and reagents have different effects upon the arteries, and whether we are to regard these experimental results as arterio-sclerosis must depend upon our answer to these questions. The subject of classification has been taken by Professor Welch; fortunately, therefore, I need not discuss the various forms. All that I need say as indicating my point of view is that I do not agree with Jore's narrower definition. His extensive studies, which have received much attention, have led him to include only a particular histological change in the vessels as coming into the category of arterio-sclerosis, while the mass of other scleroses in the arteries remains unclassified. He and those who follow him would limit the term to conditions of intimal hyperplasia, with a peculiar splitting of the internal elastic lamina, conditions which can only be distinguished under the microscope.

Are we, then, to exclude the clinician from diagnosing arterio-sclerosis? The answer can but be, No! And this for the adequate reason, that such is not the sense in which Lobstein applied the term arterio-sclerosis in 1835. Let us preserve the broader meaning, and regard all scleroses or hardenings of the arteries as included under this general term, recognizing, if need be, distinct varieties.

Thus I would point out that arterio-sclerosis is not a simple disease. Although, in some instances, a single coat of a vessel is found affected by a fibrous or other allied change, in others several tunics of the same artery are involved. Again, we may find that in a certain form of

sclerosis particular tissue elements are picked out, while other tissues are unaffected, or that when muscle fibres are degenerating in the media the connective tissue elements of the intima are proliferating. Hence we may have two or more such processes inextricably mixed in a progressive disease of the arterial walls.

Of the more common forms of sclerosis of the arteries I would point out that the hard radial vessels by which the clinician makes his diagnosis of arterio-sclerosis is a widely different disease from that recognized by the pathologist at *post-mortem* examination of the aorta. The sclerosed radial vessels represent a disease which is peculiar to the media; it has its origin in the muscle cells of the middle coat, and the middle coat alone is damaged. The intima and adventitia are not essentially involved in the process, though occasionally a secondary intimal thickening accompanies the medial degeneration. The main changes in the media are a fatty degeneration of the muscle and later of the elastic fibres, both of which become calcified. It is through these calcareous plaques in the media that the beaded character is given to the radials. At these sites of medial degeneration and calcification the vessel wall is perceptibly thinned, so that many small pouchings result. These pouchings, though small, are true aneurysms distributed irregularly in the vessel wall, and when held to the light are seen to be thin and quite transparent. This type of disease, which is most frequent in the vessels of the extremities, I shall later speak of as the Moenckeberg type of arterio-sclerosis, and I shall point out how closely some of the experimental lesions resemble it.

On the other hand, the nodular aorta, which we so frequently meet with at autopsy, is the result of repeated insults telling upon the intima alone. The thickenings of the intima may again be entirely proliferative, and in this case represent a chronic inflammatory production. This I acknowledge is not the view held by all; those who still uphold Thoma's conception of the arterio-sclerotic process see also in the typical nodose sclerotic aorta a primary giving way of the media, and regard the intimal overgrowth not so much as an inflammatory as a compensatory process. Whichever view be accepted, or be correct, or whether, as would seem to be truly the case, we encounter both conditions, it is still an open question whether the newly-developed cells in the intima are of endothelial or of connective tissue origin; it may be again that both tissues take part in the overgrowth. At all events, few or many layers of cells, which are very like endothelial cells, are produced immediately beneath the endothelium, and it was the character of these cells which led Virchow to speak of "endarteritis chronica deformans."

When a similar intimal thickening, by the proliferation of connective tissue or endothelial cells, occurs in the smaller arteries, so that the lumen of the vessel becomes distorted, or even wholly obliterated, the condition is spoken of as "endarteritis deformans sive obliterans." I may, however, mention that seldom if ever is a vessel occluded by the overgrowth of its intima alone. The usual result is that after a vessel has been partly obstructed by the thickened intima, complete blockage is brought about by thrombosis.

Now although we have ample opportunity to study the damage that has been done by the various noxæ to the human arterial system, we are as yet largely without the means of recognizing which lesion has been produced by a particular irritant. It thus becomes evident that the histological changes in the arteries must be investigated by experimental means, for it is only in this way that the changes in the arteries produced by insults of different kinds can be followed step by step, and that a decision can be reached regarding the influence of the various injuries.

It is the common fault of experimenters that, having been able to reproduce a disease in whole or in part by experimental means, the conclusion is drawn that all the features of this disease are due to this one cause. To avoid this common mistake we must advance very cautiously towards our conclusions.

THE EARLIER EXPERIMENTS.

In the earlier experimental attempts undertaken to produce arterio-sclerosis and aneurysms mechanical means were employed. Thus, Malkoff, and also Fabris, injured the vessel wall directly, either by forcibly pinching it through the skin or by laying it bare and crushing or by applying corrosive substances to its outer walls. That damages of all kinds were obtained in this manner we can readily understand, but that neither true aneurysms nor arterio-sclerosis resulted is just as clear. Thromboses and inflammation of the arteries were the most frequent results of these violent measures, but these studies have thrown little light on the process of arterio-sclerosis. Malkoff, however, made another interesting experiment, in which he laid the end of the carotid artery bare, and, ligating the vessel about an inch or so away, he put the isolated portion of the artery under artificial pressure and then returned it to its natural bed. This treatment he claimed led to a calcification of the media—a condition which he said was directly referable to the high pressure to which the vessel had been subjected.

Several authors claim to have obtained positive arterial lesions of the character of arterio-sclerosis by irritating or severing the nerves of

the leg. Of these, Lewaschew, Bervoets, and Fraenkel each described changes in the femoral and tibial arteries after performing these experiments, but in each case, as Czyhlarz and Helbing pointed out, the influence of the trophic inflammatory disturbances extending from ulcers about the limbs cannot be excluded. These experiments, thus, do not teach us more than that the coats of the arteries, including the intima, take part in an inflammatory process by direct extension from without. That the intima itself could become involved in an acute or chronic inflammation was denied by Roktitansky, who held that the thickening of this coat was the result of the organization of lymph thrown out of the blood. This contention has, however, been shown to be incorrect.

Soon after Thoma brought forward his theory that arterio-sclerosis is a compensatory thickening of a vessel in a region where the media has been weakened and the lumen of the vessel enlarged, several workers endeavoured to prove this by experimental means. Thoma, himself, undertook to show that the stimulus or irritation required for the proliferation of new tissue in the intima lay in the slowing of the blood current. By ligating a vessel he found that on the distal side of the ligature, as far as the first compensatory artery, considerable intimal thickening took place. However, Fuchs, who repeated the experiments, though he found the same changes to occur in the arterial walls, attributed the changes to a diminution of the blood pressure, while others again reported the occurrence of arterial thickening on both sides of the ligature, and ascribed its presence to local thrombosis and inflammation.

The inflammatory theory of arterio-sclerosis received further support in the experiments of Sumikawa, who irritated the vessels by painting them with turpentine or silver nitrate or infected them with bacteria. Vessels so treated showed an inflammatory condition in all the coats, or else in the intima alone. In each case there was a degeneration of the muscle fibres along with a small-celled infiltration along the vasa vasorum. His experiments with bacterial infection of the vessel walls bear out the pathological findings in man, where it is noted that inflammatory foci not only lead to a new formation of capillaries in the granulation tissue, but also of vasa vasorum in the neighbouring large blood vessels, and moreover, that the reaction in these blood vessels is accompanied by a connective tissue proliferation and thickening of the intima.

That lead, phosphorus, and mercury produce arterial lesions has long been described in medical text-books, and yet such lesions have not been produced experimentally. It is true that Lunz, in his experiments with these salts, has found that the elasticity of the vessels is dimin-

ished, but Jores could not verify these results, and was unable to find any change in the vessels of animals so treated.

THE MORE RECENT EXPERIMENTS.

Thus until 1903 little advance was made in the experimental production of arterio-sclerosis despite the many attempts. In that year Jores instituted a series of experiments at the Bonn Pathological Institute in which he fed animals on adrenalin extract, hoping thereby to test the effect on the arteries of raising the blood pressure. Whether he obtained any marked rise in the blood pressure he does not report. His results, however, on the arterial walls were negative. Josué, using the same substance, applied it in a different way. He repeatedly injected a solution into the ear veins of rabbits. After several weeks of this treatment, he found that the aorta of the animals showed distinct pathological change with aneurysmal dilatations. The lesions, which varied from the size of a pin's head to a split pea, were distributed irregularly over the thoracic aorta and over the abdominal aorta as far as its middle. The vessel changes consisted essentially of medial degenerations lying in the middle zone of this layer. The destruction of the muscle and elastic fibres with the later deposition of lime salts in them led to a thinning and weakening of the vessel wall, which later became the site of aneurysmal dilatations.

The success of Josué in producing experimental arterial lesions led immediately to like methods being employed by a large number of workers, and in the main their findings have agreed with one another and with Josué's original report. Fischer points out that the lesions produced by adrenalin are all of a medial nature, and that the process is really one of necrosis. He describes the elastic fibres lying more closely together, while the muscle cells between the elastic lamellæ are in part lost. Similar results have been obtained by Erb, Scheidemandel, Kurt Ziegler, Pearce and Stanton and others. Sturli found no difference between the lesions produced by synthetic adrenalin and the adrenalin extract. Opinion, however, remains divided as to whether we are right in comparing the experimental results in the lower animals with arterio-sclerosis as we find it in man. Some hold that the lesions are like those commonly seen in the human artery, while others again can find no similarity between the conditions.

Kurt Ziegler and I almost simultaneously compared these adrenalin lesions with the Moenckeberg type of arterio-sclerosis. We have both pointed out how in each the essential lesion is a degeneration of the muscular and elastic tissue of the media, while as a consequence

aneurysms are produced in the vessels. I have found that the degenerations in both instances are of the nature of fatty metamorphosis of the involved tissue, which later goes on to calcification.

Ziegler holds that the lesions produced by the inoculation of adrenalin are of a nutritional character, or, rather, due to lack of nutrition. Torri and others, on the other hand, regard them as the outcome of heightened blood pressure, while Fischer considers the process as a pure necrosis due to the direct action of the drug on the muscle cells. Observations in favour of the degenerative or necrotic theory have been recorded by Braun. He found that the combination of adrenalin with amyl nitrite neutralizes the pressure-raising power of adrenalin, but notwithstanding, the arterial changes manifest themselves just as when adrenalin alone is administered. A somewhat different result, however, was gained by Mironescu. He found that the inoculation of euthalmin alone had no effect on the arteries, while at the same time he noted that it produced a drop in the blood pressure. If after the inoculation of adrenalin the animals were also given a dose of euthalmin he found an initial rise with a secondary drop in the blood pressure, and that these aniamls showed arterial changes much sooner than those treated with adrenalin alone. Hence Mironescu concluded that it was the sudden change from a high to a low pressure that had a deleterious effect upon the arteries.

Harvey has recently demonstrated some very interesting experiments in regard to the degeneration of vessels. He notes that vessels under pressure undergo a more rapid destruction that those which are lax. This throws some light on the effect of high blood pressure in the arteries.

Experiments have also been undertaken by some to observe the effect of bacteria and their toxins on the vascular system. Gilbert and Lyon claim to have produced lesions similar to those produced by adrenalin.

In the main the different experimental results agree with one another, yet the inferences as to the nature of the lesions and the similarity with arterio-sclerosis in man differ widely; the majority of authors hold that the changes brought about in the arteries of animals are of an arterio-sclerotic nature, but in my opinion only one of them draws the proper inference and shows the identy of the changes in the vessels of the "adrenalin animals," and the Moenckeberg type of arterio-sclerosis in man.

THE AUTHOR'S OWN OBSERVATIONS.

It was the indefiniteness of the results obtained in the experimental work that prompted me to attempt the production of arterial lesions.

Simultaneously with Braun and Mironescu, I had conceived the idea of abolishing the high-pressure effects of adrenalin by combining it with a drug producing vasodilatation. I also observed the effect of adrenalin inoculated directly into the muscle tissue. Other substances, as digitalin and barium chloride, which have the effect of raising the blood pressure, were also tried. Lastly, the effect of producing a septicæmia with different organisms was studied, and in these experiments some interesting arterial lesions were obtained. In my series of observations over forty rabbits were used. In these animals I found that results are most easily obtained. Control animals were used in all cases where necessary, particularly in cases where the animals were inoculated with two substances simultaneously.

ADRENALIN CHLORIDE, BARIUM CHLORIDE, AND DIGITALIN.

Adrenalin chloride was administered intravenously to animals in doses varying from 0.3 c.cm. to 2.0 c.cm. of the 1 in 1,000 solution. The best results were obtained by giving large doses at intervals of three to four days. In several cases the animals died of acute œdema of the lungs immediately after the inoculation of the adrenalin, but I have never met with a case of death from cerebral hæmorrhage, as has been reported by others.

The arterial lesions varied in extent and severity with the length of time the animals were under treatment, with the quantity of adrenalin inoculated, and with the idiosyncrasy of the individual animals. The lesions were usually present at the end of two or three weeks, and the early changes consisted in small isolated plaques of calcification with pittings in their centres. When these grew larger, saccular aneurysms made their appearance. These lesions were distributed mainly over the thoracic aorta and in the abdominal aorta as far as the renal vessels.

Again, in other cases it was found that the entire thoracic aorta, half of the abdominal aorta, the vessels of the neck and those of the abdomen were completely calcified. The thoracic aorta, however, alone showed a diffuse aneurysmal dilatation, beginning at the aortic opening and reaching as far as the diaphragm. Neither the abdominal aorta nor any of the smaller vessels were involved in this dilatation.

The results obtained by the inoculation of barium chloride were exactly the same as those produced by adrenalin; in fact, the similarity is so striking that the lesions cannot be distinguished from one another either macroscopically or microscopically. In several cases I was able to produce the diffuse aneurysm of the aorta by the use of barium chloride, and in each example it was striking how the aneurysm was

isolated to the thoracic portion of the vessel and did not advance beyond the diaphragm.

Fischer's experiments, too, of producing arterial lesions by the intravenous inoculation of digitalin, were also repeated, and I agree with his findings that the arterial lesions isolated in the aorta are similar to the milder adrenalin destructions.

It was further found that, if the pressure-raising effect of adrenalin be abolished by the use of nitroglycerin, although the arterial lesions were not as extensive as when adrenalin alone was used, nevertheless, tissue degeneration in no way differing from that produced by adrenalin did still occur in the vessel walls.

In such cases where the arterial lesions were just beginning there was change to be noted in the vessels macroscopically. I might point out, too, that in none of the vessels that I have obtained from animals treated with adrenalin was I ever able to make out any naked eye changes in the intima. This coat was at all times stretched smoothly over the damaged media. The earliest damage was always found in the muscle cells of the middle zone of the media. Here patches of homogeneous tissue were met with, where the muscle nuclei were lost, but where the elastic fibres passed through these areas unaffected. With the loss of the muscle cells the parallel elastic fibres were crowded closely together by the blood pressure within the vessel.. This crowding of the elastic fibres from within outwards naturally led to a small dimple at this point and this was the beginning of a saccular aneurysm.

The loss of the muscle cells takes place by a form of necrosis, as was pointed out by Erb and Fischer. The elastic fibres later become affected, losing their elasticity and contractile power. This degeneration of both muscle and elactic fibres occurs through a process of fatty change, which is in some cases difficult of demonstration, but which is, however, readily brought out out in those cases where the metamorphosis is slower. With the high calcium content of the rabbit's blood these areas of fatty degeneration in the media of the aorta and other vessels are converted into calcified plaques by the process described by me elsewhere. Microscopically, no connexion could be linked between the positions of the vasa vasorum and the arterial degenerations, and a true mesarteritis, as noted by Fischer, was not met with.

In no instance have I found a primary change occurring in the intima after any of the above treatments, though in one or two specimens I did note the slight thickening of the intima at the margin of the aneurysm.

It is to be noted, too, that with the abolition of the physiological effects of adrenalin, the arteries are still affected, though more slowly

and to a less degree than where the vessels are under tension. Boveri claims to have abolished the effects of adrenalin on the blood vessels by combining it with "Iodipin," though he was not able to prevent the toxic effect on the muscle cells.

The effect of adrenalin chloride inoculated directly into the skeletal muscle depends upon the strength of the dose given. When the undiluted 1 in 1,000 solution of adrenalin chloride is inoculated into the muscle tissue the cells are killed outright, so that the nuclei and cell membrane disappear. Weaker solutions produce a fatty degeneration of the muscle cells. It was found also that the animals receiving the adrenalin treatment over an extended time developed fatty degeneration of the heart. So we can but conclude that adrenalin has a selective action on muscle tissue, and that its toxic effect thereon is the primary cause of the arterial lesions. The same holds true for barium chloride and digitalin. The three substances are thus similar in their effects, differing only in the intensity of their reaction.

The influence of high pressure in producing arterial change is well brought out in these experiments. We have noted that the most frequent site and the most severe changes occur in the thoracic aorta, and that the vessels in the remote parts of the body are only affected when advanced lesions are present there. We must admit that the inoculated substances are distributed equally to all parts of the body, and that from toxæmia alone all vessels of similar structure should suffer equally. But the normal amount of work done, besides the increased strain that is produced by raising the blood pressure, is felt most severely in the aorta, mainly in the thoracic portion. As a result of this combined degeneration and high pressure, the thoracic aorta exhibits a fusiform aneurysm, extending from its origin to where it passes behind the diaphragm. From this localization of the diffuse aneurysm to the thoracic aorta, it is evident that the aorta opening in the diaphgragm acts as a flood-gate in letting through only given quantity of fluid. By this mechanical device the abdominal aorta is relieved of having an increased volume of blood thrown into it by the overworked heart, and thus is not subjected to the double degenerative forces of toxæmia and high blood pressure, as is the thoracic portion. Focal degenerative lesions are neverthless found in the abdominal aorta.

The important role that the muscle fibres of the media play in the strength of the arterial wall is well known. In fact, it is pointed out that they are the mainstay of the vessel. This fact is exemplified in these experiments, where it is found that with the primary degeneration in the muscle cells the vessel wall begins to give way in this region. The

elastic fibres at this time, though themselves not visibly altered, no longer take on the wavy contour which is characteristic of them in a relaxed vessel. It would seem from this that the apparent elasticity, as shown by their undulations, is not an inherent quality, but is due to the contraction of the muscle fibres surrounding them—or, otherwise, that when the artery is in a condition of tonus, its contracted state is due mainly, if not entirely, to the muscle fibres; when dilated it is probable that the elasticity of the elastic fibres comes into play.

A proliferation of the intimal tissue in these cases is to be regarded as secondary to degenerative processes in the media. The proliferation is either of the character of a hypertrophy of the musculo-elastic layer or of the subendothelial tissue. Whether this subendothlial tissue had its origin in connective tissue or endothelial cells we cannot discuss here.

INFECTIVE ARTERIO-SCLEROSIS.

I have also undertaken the production of experimental arterial lesions with infective agents. For this purpose B. typhosus and streptococcus were used in separate experiments, while again in others diphtheria toxins were inoculated. Each of these agents was inoculated intravenously into rabbits.

The results obtained with B. *typhosus* and the streptococcus were of the same order. The first part of the pulmonary artery and the ascending limb of the aorta showed warty thickenings of the intima. There were no anerysmal sacs nor any sign of a calcareous degeneration of the media. Microscopically there was a fatty degeneration of the subendothelial tissue, while there was, however, much connective tissue advancing into the degenerated area. A small-celled infiltration was wanting, as was also any sign of calcification. At the areas of thickening of the intima it was found that the internal elastic lamina had split into several parallel layers, which were stretched between the proliferating cells. The area affected included the intima and the inner layer of the media. Thus we find that these infective lesions (of *B. typhosus* and streptococcus) differ entirely from those produced in our adrenalin series. What we may term the adrenalin group are agents producing destruction of tissue leading to a calcification with little or no local repair to make up for the lost tissue, while the mild infections lead to a slight degeneration of the vessels coats, though the process is followed step by step by the process of repair, and instead of getting a thinning of the vessel wall there is an actual thickening. It becomes self-evident that with the absence of extensive destruction of the muscle fibres in the media no aneursyms were formed.

In our experiments it must be remembered that we dealt with cultures of low virulence. The possibility must not be overlooked that virulent micro-organisms gaining entrance in to the adventitia through the vasa vasorum, and proliferating there, might invade the media and induce local degeneration and destruction, and if the reparative process could not keep pace the weakening of the media might result in aneurysm formation. Such lesions would correspond to the mitotic aneurysms in man, which have been described by McCrae and others; nay more, the observations of Heller, Chiari, and others upon syphilitic mesaortitis, afford a like explanation for aneurysms in the syphilized.

The presence of lesions in the pulmonary artery is worthy of note in comparing the distribution of the lesions with those of the adrenalin series. In the later, the aorta and its branches were alone involved, while the heart became hypertrophied—a feature that was not seen after the bacterial inoculations.

The repeated inoculations of diphtheria toxin into rabbits gave surprising results. Here, instead of meeting with proliferative changes, such as the B. typhosus and streptococcus produce in the aorta, there were only lesions of a degenerative character. The degenerations were isolated to the first part of the aorta, and were identical with those produced in the adrenalin series. The thinning of the arterial wall, with calcification and aneurysmal dilations, were all present, and the microscopical examination showed the lesions to be confined to the media. No proliferative or inflammatory changes were present in the intima, nor was there any change about the vasa vasorum.

Hence we have before us two interesting groups of arterial lesions resulting from infective conditions. On the one hand, lesions are intimal and proliferative, while on the other they are of a purely medial degenerative nature. The free toxins of diphtheria have a predilection for the muscle tissue of the circulatory system, whereas the endotoxins of typhoid and streptococcus infections are in small doses rather of a stimulating nature to the connective tissue and endothelial cells.

If, then, we are to consider the nature of the lesions produced in the arteries as a criterion in classifying the toxins, we must place the diphtheria toxin along with the adrenalin series, while the endotoxins, the stimulating or proliferative agents, form another. The marked differential characters which are brought out by the two series in experimental animals make it more than probable that such differences also exist in man—that is, that typhoid or streptococcus infection will lead to an endarteritis, while diphtheria will produce lesions of a degenerative character, affecting chiefly the muscle cells.

The fact that the streptococcal and typhoidal infections lead to a splitting of the internal elastic lamina with a proliferation of the sub-endothelial tissue (and also the musculo-elastic layer) places the lesion in very close relationship with arterio-sclerosis in man, as it is described by Jores.

To sum up the results of my experiments, I find that:

1. The effect of the high-pressure drugs (adrenalin chloride, digitalin, and barium chloride) on the arteries is a degenerative one, as was described by Fischer and Erb for adrenalin.

2. The muscle cells of the media are first attacked, while the elastic fibres of this layer are also involved later.

3. At a proper stage of the degeneration, a fatty change can be demonstrated in the tissues, followed by calcification.

4. The middle zone of the media is always involved.

5. Occasionally secondary reactions occur in the intima which are of a proliferative nature.

6. The effect of adrenalin is not abolished by lowering the blood pressure with nitroglycerin.

7. The aneurysms are produced as a result of the destruction in the media.

8. These experimental lesions are in every respect similar to the Moenckeberg type of arterio-sclerosis.

9. The effect of diphtheria toxins on the arteries is similar to that of the adrenalin series.

10. Typhoid and streptococcus infections produce little destruction of tissue cells, but tend to stimulate cell proliferation in the intima and inner layer of the media.

11. Vessel changes are brought about by these infections which correspond to arterio-sclerosis, as described by Jores.

12. Contrary to the general conclusions reached by Thoma, these experiments show that there is definitely a form of arterio-sclerosis in which, not a preliminary weakening of the media, but a primary proliferation of the intima, including the musculo-elastic layer, is the prime feature. To what extent this essentially proliferative type is to be encountered in the human aorta and other vessels must be left an open question. Undoubtedly in the medium-sized arteries, the Moenckeberg type of medial degeneration is common. Undoubtedly also in syphilitic as well as other cases, we encounter in the aorta a secondary and adaptive or compensatory over-growth of the intima—secondary that is, to the medial degeneration.

Bibliography.

Boveri : *Deut. med., Woch.*, 1906, p. 877.
Braun : *Münch. med. Woch.*, 1905, p. 533.
v. Czyhlarz and Helbing : *Centralbt. f. Path.*, 1897, vol. viii, p. 849.
Erb: *Archiv f. exper. Path.*, 1905, vol. liii, p. 173.
Fischer : *Zeits : f. Psychiatrie*, 1904, vol. lxii, p. 241 · Verhand. d. Kongress f. innere Med., 1905, vol. xxii, p. 235 : *Deut. med. Woch.*, 1905, p. 1713.
Gilbert and Lion: *Archiv. de Méd. Expér.* 1904, vol. xvi, p. 73. *Comptes Rendus de la Soc. de Biologie*, 1889, p. 583.
Harvey : *Journ. of Exper. Med.*, 1906, vol. viii, p. 388.
Josué : *Presse Médicale*, November 18th, 1903; *Journ. de Physiol. et de Path. Gén.*, 1905, vol. vii, p. 690.
Jores : *Ziegler's Beiträge*, 1902, vol. xxxii, p. 146.
Klotz : *Journ. of Exper. Med.*, 1905, vol. vii, p. 633 ; 1906, vol. viii, p. 322.
Lunz : Inaugural Dissertation, Dorpat. 1892.
McCrae : *Journ. of Path. and Bacter.*, 1905, vol. x, p. 373.
Mironescu : (Ref.) *Centralbl f. innere Med.*, 1906, vol. xxvii, p. 598.
Pearce and Stanton : *Trans. Assoc. of Amer. Phys.*, 1905, vol. xx, p. 513.
Sumikawa : *Ziegler's Beiträge*, 1903, vol. xxiii, p. 242.
Scheidemandel : *Virchow's Archiv*, 1905, vol. clxxxi, p. 363.
Sturli : *La Semaine Méd.*, 1905, vol. xxv, p. 128.
Torri : *Centralbl. f. Path.*, 1906, vol. xvii, p. 319.
Ziegler, k. *Ziegler's Beiträge*, 1905, vol. xxxviii, p. 229.

DISCUSSION.

Dr. Pearce said experimental lesions were not analogous to those of man, but of great value in explaining degenerative and regenerative changes in vessels. Physiological studies of the action of adrenalin were of great value in explaining problems of cardio-vascular pathology.

Professor Clifford Allbutt said: I must begin my remarks, Sir, by thanking you for the compliment you pay me in calling upon me, who am no expert in pathology, to speak in this Section. The discussion of this morning is peculiarly instructive and gratifying to me in so far it has come to the support of the doctrine in which for so many years I stood alone—the doctrine of the mechanical origin of a certain large group of cases of arterio-sclerosis, a group which includes that of chronic renal disease, and especially of granular kidney, but is by no means confined to cases of renal disease. And although it is true that mechanical causes, which we may express pretty nearly in terms of arterial blood pressure, operate in all cases of arterio-sclerosis, of the group of which I am now speaking it is virtually the sole cause; in other words, the arterial damage is due, and stands in proportion to a period or periods of excessive pressures, an excess which, in the first instance, is antecedent to the arterial disease, and may even within some such term as four or five years be subdued, and the arterial damage thus averted—an opportunity which we must be ever more and more on the alert to seize and to turn to advantage. But this mode of arterial disease, the mode which I have

called that of hyperpiesis, is by no means the only one. Speaking as a physician, I recognize two other classes, at least, of arterial disease—classes which I have named respectively the toxic and involutionary classes—each of great extent and importance. In these classes the arterial pressure are not characteristically high. Tension or strain (which, of course, cannot be excluded) is not the prime factor in setting up the arterial disease. In these two classes, indeed, the arterial pressures often run low, and—intercurrent contingencies apart—do not exceed the average for the time of life of the individual. The mechanical wear and tear would be harmless but for some causes of other kinds which produce in the vessels a morbid liability to yield under ordinary stresses. Now it is here that as a physician I come to the pathologist to inquire if, in accordance with these several clinical features, he can separate arterio-sclerosis into corresponding histological varieties? I would suggest—and my own cruder efforts in pathology lead me to think —that in the arterial disease of toxic origin the poisonous agent enters by the adventitia and vasa vasorum, whence it penetrates to the intima, but on its way leaves the adventitia and the media not without traces of its deteriorating influence; that in the arterial disease of involutionary origin the disease begins early, if not first in the media, by spots and streaks of decay going on to calcification; that in the hyperpietic disease the damage begins by tearing and shearing stresses, by shears, or even by rents, which, indeed, do not always elude the microscopical eye. These shears act chiefly in the plane *minoris resistentiae*—the plane of opposition of the intima and the media. When the rents are manifest to the eye, they lie tangentially to this plane, and the elastica is fragmented, or is reduced to common fibre. Such, however, is the consequent obliteration of normal landmarks that we know too well how difficult it is to be topographically exact in these descriptions. From such considerations as these I have urged that the clinician should discard the name of arterio-sclerosis as the name of a disease, and be content to speak of arterial disease as of several kinds, or, at any rate, as a result of many maladies, maladies which are divisible between at least three different classes. With what reserves and distinctions the pathologist is to use the term it would not become me to discuss before this audience. In addition to the surmises I have just ventured upon, I may, however, propose one more consideration, namely, that in arguing upon the relative meanings of arteritis, arterio-sclerosis, atheroma, and so forth, we must constantly bear in mind the stage, the rate, and the place of the process in each particular case. Different as such processes

may be—as indeed I have urged—yet we are apt, I think, at times to forget that in comparing tissues from a very slow case with those of a case whose course has been quicker, or in comparing, in the same case, a muscular artery with a piece of the aorta, or, again, in comparing an earlier stage of disease in a patient cut off by contingent causes with a later or final stage in another patient, we may in the differences of time and place overlook essential similarities or even identities. May I say, in conclusion, a few words on arterial hypermyotrophy? Whether there be in arterio-sclerosis a true hypertrophy of the muscular coat is a fallacious question, if so be that arterio-sclerosis is a general term including several kinds; for this hypertrophy may occur in one or more of the kinds, but not in all. This, in my opinion, is the case: arterial hypermyotrophy occurs in the kind of arterial disease I have called the hyperpietic—that in which the disease is a result of excessive pressures within the vessels, but does not occur in either of the kinds which I have called toxic and involutionary; unless, perchance, their ordinary course is modified by some incidental burden of blood pressures. Leaving these two kinds, then, and turning our eyes to the arterial disease of high pressures only, we shall find if time be given, the first deviation from the normal to be this hypermyotrophy. At this stage, if the abnormal pressures be permanently reduced within normal limits, the hypermyotrophy will disappear also. Such transitory phases are best witnessed in young, or comparatively young, subjects. If, on the contrary, excessive pressures persist, unrelieved by nature or art—say for more than four or five years—this hypertrophied coat seems to lose its specific quality and to deteriorate into fibre of inferior rank, but perhaps capable of more tenacious resistance. This process is deferred, I think, in small arteries in which constriction has been especially dominant, a constriction whereby the pressure within them and the consequent tension of their coats are reduced. This vascular hypermyotrophy was described first by George Johnson, and Dr. Savill and other recent observers have verified rather than enlarged his description. It fell to me, perhaps to point out that this hypertrophy of the media is not confined to granular kidney, but arises in all cases of continuously high arterial pressure—all cases, that is, in which these parts are nutritionally capable of re-adaptation. Gull and Sutton erred, as I pointed out in a contemporary number of the *British and Foreign Medico-Chirurgical Review*, not in describing other kinds of pathological change in the arteries but in doing so to the exclusion of Johnson's kind. The problem in this hypermyotrophy now to be decided is whether spasm of the vessels concerned, under the influence, let us say, of some poison

acting upon them directly or indirectly through the vasomotor centre, suffices to produce it, or, as I have ventured to urge, that it is due in the vessels, as in the heart, to enhanced dilating stresses. The answer is not easy, as the closer the constriction in any vascular area the lower must be the pressure, which, *caeteris paribus,* is converted into velocity. Perhaps if the resistance distal to the area of constriction be high, spasm and high internal pressure may co-operate to produce hypermyotrophy. That the muscular arteries on the hither side of areas of spasm will dilate and hypertrophy under the rise of pressure caused by it needs, I think, no asseveration. And how, thereafter, under this strain, arterial disease arises—hypermyotrophy is scarcely to be called disease—I have already stated; this is, of course, a later phase, a phase in which the state of the vessel passes into the irremediable. But for a moment I may return to calcification. Calcification rarely occurs in the arteriosclerosis originating in high pressures; it is characteristic of the involutionary kind. But it is a common error to suppose that calcification is a very slow process, or one confined to old age. It may scatter itself widely and profusely in comparatively short periods, and it may attain even extreme degrees so early as the fifth decade of life, possibly in rapidly decaying individuals, even sooner. On the whole, then, calcification is, clinically speaking, a presumption against hyperpiesis, present or past—hyperpiesis, that is, above the degrees usual for the time of life. Notwithstanding, I have records of a few cases, primarily of hyperpiesis, observed over long periods of time, in which calcification supervened—exceptions which test the rule. For in these it was apparent, on consideration of all the facts of each case—facts clinical as well as pathological—that the calcification appeared when the proceses of hyperpiesis had ceased or become subordinate, and the life of the patient had been spared to undergo the ordinary involutionary changes which are present in the vast majority of elderly persons.

Professor Adami said: While appreciating fully the distinction which Professor Clifford Allbutt has drawn between compensation and adaptation, I feel bound as a pathologist to cross blades with him regarding the importance and the frequency of adaptive conditions; nay, more, I would go so far as to lay down that pathological processes so far as they are reactive are coincidently adaptive to a very great extent. As pathology widens itself from a study, histological and otherwise, of morbid states, to one of morbid processes, so inevitably are we driven to realize that this is so. I need but recall that the abundant and valuable recent studies upon acquired immunity, upon hæmolysins, cytolysins, and the like, recall to us a vast series of these adaptations. And here,

in connexion with the ordinary type of arterio-sclerosis, it has for long seemed to me that we encounter some of the most striking instances of adaptation. The studies made by me some years ago, to which I have referred in the Middleton-Goldsmith Lectures of 1896 upon Fibrosis and Inflammation, led me to support and confirm Thoma's contention that in the commonest type of aortic arterio-sclerosis there is (as in the later experimental researches of Josué, Pearce, Klotz, and so many other workers with adrenalin and allied drugs) a primary degeneration and giving way of the media. To that view, despite Jore's important studies, I still incline, and would harmonize the divergent opinions by laying down that while the primary lesion manifests itself in the media, the primary reaction to that lesion occurs in the intima. It is this reaction—this overgrowth of the musculo-elastic layer—that Jores has so serviceably brought to our notice, an overgrowth which may or may not be accompanied by coincident hyperplasia of the subendothlial connective tissue of the intima. No one nowadays regards this intimal hyperplasia as strictly inflammatory, as a direct reaction to injury; it has none of the characteristics of inflammatory new growth; there is no primary new formation of vessels, no small-celled infiltration; it is a hyperplasia pure and simple, occurring not in the tissue primarily injured, the media, but in another tissue, the intima. But it is secondary to the giving way of the media, and to that extent reactive, and can, I think, only be regarded as adaptive, tending towards a restoration of the original lumen of the vessel and a strengthening of the wall at the region of giving way. I have elsewhere explained this overgrowth as an effect of strain within certain limits. Just as increased strain, up to a certain grade, favours muscular hyperplasia, and as exercise, by causing increased pull upon the tendinous insertions of the muscles is followed by overgrowth of the bony ridges of attachment of those tendons, so I hold that when the media gives way locally to a slight extent the overlying intimal tissue becomes stretched and strained, and as a result exhibits hyperplasia. Too severe and sudden an expansion of the intima arrests any such tendency to overgrowth. Thus, it is significant that in aneurysms intimal thickening is lacking. There is a similar lack of overgrowth in these aortas from rabbits treated with adrenalin and barium chloride, with their aneurysmal pouchings, as, again, in the pouchings of human iliac and femoral arteries of the Moenckeberg type. I shall not be surprised, however, if further observations demonstrate that the common or Jore's arterio-sclerosis and the Moenckeberg or medial degenerative type are the effects of common noxæ acting with

different grades of intensity, and, as Professor Aschoff suggests, upon arteries of different constitution. I am inclined, that is, to think that the experiments made so far with adrenalin have been somewhat severe, leading to an acute type of medial degeneration, and that slighter grades of intoxication conducted over long periods will afford the reactive overgrowth of the intima, such as we see in human arterio-sclerosis of the ordinary type; indeed, from Dr. Pearce's published description of certain of his experiments, which tended to fulfil these conditions—experiments in which he gained not merely medial giving-way, but also intimal hyperplasia—it would seem that this relationship between the two types is in a fair way to receive its proof. While saying this I need scarcely add that, as demonstrated by Dr. Klotz's most valuable observations upon the effects of bacterial toxins on the aorta, there is a wholly different type of sclerosis in which we have to recognize a primary intimal overgrowth after the type of chronic endarteritis proliferans, as also yet another important type, as demonstrated by the Kiel school and Chiari, that following upon a chronic mesaortitis—the syphilitic type.

[The remarks of PROFESSOR ASCHOFF, who led the discussion, given in German and not reported by the British Medical Journal, were to the effect that there is perhaps too great a tendency to regard the arteries in general as of like structure. This is far from being the case: there is an essential relationship between function and structure and the function of the arteries of different regions varying we must be prepared to find, and, in fact, do find, that there is marked variation in the relative development of the different coats. From this it follows that one and the same noxae must be expected to have different effects upon different arteries, while certain noxae may attack one group of arteries and not others. We must be prepared to find in the future both that what appear to be distinct lesions in different parts are due to the common cause, as conversely that distinct noxae act specifically upon particular arteries setting up distinct forms of arteriosclerosis.]

[Reprinted from the Boston Medical and Surgical Journal,
Vol. clvi, No. 9, pp. 267–269, Feb. 28, 1907.]

EXPERIMENTAL STUDIES IN ARTERIOSCLEROSIS.*

BY OSKAR KLOTZ, M.D.,
Resident Pathologist, Royal Victoria Hospital, and Lecturer in Pathology, McGill University, Montreal, Canada.

Our conception of the pathology of arteriosclerosis has recently been so altered, that much confusion exists at the present date, as to what form or forms of arterial disease should be considered under this term. There were and are still many who would limit the term to a single kind of lesion, while others again are more liberal, and use this appellation to include the great mass of arterial diseases which eventually lead to a thickening of the vessel walls. The author of the term leaned towards using the word "arteriosclerosis" for all conditions of hardening of the arteries, and with this meaning the old anatomists expressed themselves, reserving, however, the term "atheroma" for another distinct lesion. However, Virchow's description of endarteritis chronica deformans, as the commonest type of arteriosclerosis, has led to the adoption of these expressions interchangeably, and has found the most favor recently. In my own studies I have given the wider use of the word, and include under arteriosclerosis, as was formerly the case, all hardening of the arterial coats. This, you will agree, is more in accord with the findings of the clinicians, for, *intra vitam*, they can in no wise differentiate the various histological forms of arteriosclerosis. It is necessary to bear in mind

* Read before the Medical Association of the Greater City of New York, Jan. 21, 1907.

this wide definition I have given to the term, for otherwise it may be held up to me that arteriosclerotic lesions were not obtained in the experimental work.

One must also not lose sight of the diverse functions which the vascular system has to perform, and how the manifold work of the arteries is at the bottom of their histological structure. The arteries of the uterus, which are well supported by the muscle tissue of the organ, are rich in muscle fibers, while the quantity of elastic fibers falls much in the background. These uterine arteries require this abundant muscular development for the periodic congestion at menstruation and pregnancy. On the other hand, the splenic arteries which within the organ have a poor supporting framework must rely entirely on their own strength to withstand the blood pressure. This extra strength of the arteries is obtained in a well developed adventitia, strengthened by elastic fibers.

The intima, too, of the different arteries varies in its constituents. Thus the arteries of the first order possess a muscular layer in the intima which is not present in the smaller arteries. Certain diseases therefore can and do occur in the intima of these larger vessels which can in no way be developed in their branches.

The experimental production of arteriosclerosis in animals is of fairly recent origin. The first experiments undertaken were by the direct injury, as crushing of an artery. In this way the experimenters had hoped to bring about sufficient change in the vessel walls to lead to aneurism. They were disappointed in this, but instead of an aneurism they found that certain local inflammatory changes with endothelial proliferation were produced. It has since

been shown in all cases where an artery is disturbed in its natural bed, thereby affecting the vaso vasorum, that an inflammatory reaction is the result.

From a study of these inflammatory processes two important facts were noted. Firstly, that an inflammatory reaction in the media is evidenced by a leucocytic infiltration about the vasa vasorum and in the lymphatic channels; and secondly, that a lesion of the media of inflammatory nature may lead to a chronic proliferation in the intima. This intimal proliferation is the result of endothelial and connective tissue overgrowth and is similar to the disease which Virchow called " endarteritis chronica deformans " in man, while the medial inflammation is like Koester's " mesarteritis." This mesarteritis passes gradually from the acute inflammatory stage into the process of chronic healing in which fibrous tissue is laid down in the middle coat of the artery. In man the most severe form of this disease is met with in syphilitic arteritis, but no doubt other infections can lead to the same results.

Since the above mechanical experiments were made, several other ways were found to bring about the same results. The chronic endarteritis has been brought about by the intravenous inoculation of bacteria of low virulence. Thus I have been successful in producing an endarteritis chronica deformans in the arch of the aorta and sometimes in the abdominal portion by the injection of old laboratory stocks of the streptococcus or B. typhosus. A true inflammation of the media (a mesarteritis) I have not succeeded in obtaining except when an injury had been induced close to the vessel itself. In this case the inflammation of the surrounding tissue spread into the arterial wall.

The experimental endarteritis chronica deformans has histological characters quite similar to that in the human arteries. The lesion is composed of a heaping up, layer by layer, of the endothelial cells, while the connective tissue underneath the endothelium is also undergoing a proliferation. The result is that a white, pearly plaque is produced, under which degenerative changes of a fatty character may develop in the deeper part of the intima.

Whether these pearly plaques in the human or experimental arteriosclerosis are composed wholly of endothelial cells, or of connective tissue, or both, is quite immaterial at present. The result is the same, the production of a nodular hyaline mass of tissue on the surface of the intima. Such a thickening of the intima has a disastrous effect on the tissue just underneath it. The intima and the inner third of the media derive their nourishment from the lumen of the vessel, and the production of a firm mass of tissue at one point in the intima cuts off the supply of nourishment to the cells underneath it. From this there follows the fatty change in the deep layers of the intima and the inner portion of the media, a condition which is so often seen in the aorta.

The experimental lesions which have of late received the most attention are of a different nature. I have just pointed out that the endarteritis chronica deformans is essentially of a proliferative character, and that degenerative processes, if they occur at all, are secondary to this. We have, however, on the other hand, been able to produce pathological conditions which from the first are degenerative in nature. By the use of adrenalin, digitalin, nicotine and barium chloride, it has been shown that *the muscle cells in the middle zone of the media are*

primarily attacked, and according to the intensity of the intoxication of these drugs, the cells either undergo a fatty degeneration or complete destruction. Along with the death of the muscle cells, the elastic fibers in the media are also affected and, like the former, they either become fatty, or with more severe intoxication, undergo necrosis. However, in each instance the muscle fibers are primarily affected. These lesions, it is obvious, have destroyed the most important tissues in the artery, and have weakened the vessel wall very considerably. Aneurisms are commonly to be found at the sites of medial change while little if any intimal compensation occurs. Thoma's dictum, therefore, that intimal compensatory hypertrophy follows medial weakening is not universally true. This type of arterial disease, in which the media is first destroyed, is spoken of as "Moenckeberg's arteriosclerosis."

Not alone was the medial degeneration with calcification produced by means of drugs, but I have also obtained it by the inoculation of the diphtheria toxin. This is important in demonstrating that the effects of diphtheria are not confined to nervous tissue and heart muscle, but that the muscle elements of the vascular system are also attacked. It may be that the intoxication in cases of diphtheria is an important agent in bringing about Moenckeberg's arteriosclerosis, such as is seen in the radials and other peripheral vessels.

This latter form of medial degeneration with aneurismal pouchings has also its analogy in the peripheral arteriosclerosis in man. The greater majority of the cases of arteriosclerosis which are diagnosed from the condition of the radial arteries are of this type. The beadings so often noted in the radials of old people are the small pouchings in the vessel wall that have become calcified.

It is therefore evident that, if the term arteriosclerosis is to be retained for the use of the clinician, this form of arterial disease, which is most commonly seen at the bedside, must be included under it.

The calcified plaques of the aorta or the calcareous beadings in the radials and other peripheral vessels are in each case secondary to a previous fatty change of the tissues. Experimentally these deposits of lime have been produced in connection with the medial destruction, when both muscle and elastic fibers become fatty. It is interesting to trace the course of the muscle tissue through the process of fatty degeneration with a subsequent death of the cells. The fine fat droplets within the cells are converted into the fatty acids by the lipase of the blood and serum, following which the salts of lime form a stable compound with the fatty acid in the form of lime soaps. These fatty acids and soap compounds are readily demonstrable by special staining. From the calcareous soaps the phosphate and carbonate of lime become deposited later.

Thus, up to the present, we have at our command the production of three types of arteriosclerosis, namely, (1) endarteritis chronica deformans, (2) mesarteritis, (3) Moenckeberg's type of arteriosclerosis. Each of these experimentally produced arterial diseases follows the same course and has the same ultimate result as in man. However, as the lesions are produced in healthy animals, which have power to compensate the effect of extreme arteriosclerosis, fewer symptoms are to be noted. The heart rapidly becomes hypertrophied and is able to carry the new load with comparative ease.